IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Cagle, et al.

Serial No.: 10/715,055

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Examiner: Fay, Zohreh A.

Group Art Unit: 1614

For: METHOD OF TREATING OPHTHALMIC INFECTIONS WITH MOXIFLOXACIN COMPOSITIONS

DECLARATION OF DAVID W. STROMAN, Ph.D. UNDER C.F.R.§1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
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Sir:

1. I, David W. Stroman, declare as follows:

2. I am the Director of Anti-Infective Microbiology for Alcon Research, Ltd. ("Alcon"). I have been employed in this capacity since August of 1990. Prior to that date, I was affiliated with various research efforts in the biomedical field, including infectious disease research. I was awarded a Ph.D. in Biochemistry and Molecular Biology by the University of Oklahoma Medical School in 1970 and performed postdoctoral studies in the Department of Microbiology and Immunology of Washington University School of Medicine from 1970 to 1972. Further details regarding my educational background and experience in the field of biomedical research are provided in my Curriculum Vitae, a copy of which is attached as Appendix A.

3. I have been responsible for all of Alcon's research programs in the field of ophthalmic anti-infective products since 1990. As a result of this experience, I have become very familiar with the state-of-the-art relative to the prevention and treatment of ophthalmic infections, particularly infections caused by pathogenic bacteria and viruses.

4. The microorganisms that cause disease in humans (i.e., "pathogens") vary from one part of the body to another. Consequently, the bacteria that are considered to be pathogens relative to the eye may not be pathogens in other parts of the body and *vice versa*. For example, *Staphylococcus epidermidis* is a major pathogen in endophthalmitis, which is a serious, sight-threatening ocular infection, but is part of the normal flora on human skin. Another pathogen that can lead to serious ocular infections (as well as other infections) is *Pseudomonas aeruginosa*, which is a Gram negative bacterium. Ocular infections involving *Pseudomonas aeruginosa*, if left untreated, may cause a patient to lose their vision and perhaps even the affected eye itself.

5. I am a co-inventor of the invention claimed in the pending United States patent application captioned above (the "pending application"). The invention resulted from a research program at Alcon that was directed to the discovery of new ocular anti-infective compositions that would be more efficacious than Alcon's existing product line in the mid-1990's. That product line included TOBREX®, which contains tobramycin, an aminoglycoside antibiotic, and CILOXAN®, which contains ciprofloxacin, a second generation fluoroquinolone antibiotic. My objectives in directing this research program were to identify new anti-infective agents that had: (a) a broad spectrum of activity against ocular pathogens, particularly with respect to isolates of ocular pathogens that had developed resistance to the fluoroquinolones utilized in existing ocular anti-infective products (e.g., ciprofloxacin); (b) greater potency than either tobramycin or ciprofloxacin; and (c) superior ocular bioavailability, relative to ciprofloxacin and ofloxacin, which represented the state-of-the-art in ocular fluoroquinolone anti-infective therapy prior to our invention.

6. Alcon evaluated many compounds, including fluoroquinolones, before selecting moxifloxacin for its next generation ocular anti-infective product. As discussed below, this selection was based on unique properties of formulated moxifloxacin, particularly its superior ocular bioavailability.

7. I have reviewed the following materials in connection with the preparation of this Declaration: (a) the Office Action from the United States Patent and Trademark Office on the pending application bearing a mail date of April 4, 2007 (the "Office Action"); and (b) U.S. Patent No. 5,607,942 (Petersen, et al), WO 90/01933 (Cagle, et al.) and U.S. Patent No. 5,597,560 (Bergamini, et al.). It is my understanding that the Examiner is asserting that the invention claimed in the pending application is not patentable in view of these prior publications. For the

reasons expressed below, I believe that the invention claimed in the pending application is clearly not obvious in view of the foregoing references.

8. The ocular bioavailability of moxifloxacin following topical application of an ophthalmic moxifloxacin composition to the eye is superior to that of other fluoroquinolones. As discussed below, the superior ocular bioavailability of moxifloxacin, when administered via an ophthalmic composition, has been demonstrated via numerous scientific studies. Such studies have been conducted both by Alcon scientists and others engaged in ophthalmic anti-infective research.

9. In column 58 of the '942 patent, *in vitro* data (i.e., minimum inhibitory concentration or "MIC" values) for 18 fluoroquinolone compounds is presented. The MIC values for the 18 compounds are compared to the MIC values for the second generation fluoroquinolone compound ciprofloxacin. The data presented in column 58 of the '942 patent are of relatively little value to a person searching for improved ocular anti-infective therapies for at least the following reasons:

(a) The spectrum of bacteria utilized in the testing is not the same as that typically utilized to evaluate anti-infective agents for possible use in preventing or treating ophthalmic infections. In particular, there is no data for *Pseudomonas aeruginosa*, which is the most dangerous ocular pathogen, relative to infections that may cause a loss of sight.

(b) There is also no data for bacteria identified as quinolone-resistant, i.e., bacteria that have developed resistance to prior quinolones used in ophthalmology, particularly ciprofloxacin and ofloxacin, such as quinolone resistant *Staphylococcus* species. Consequently, it is not possible to determine if the compounds identified in the '942 patent would be any more effective against such bacteria than existing fluoroquinolones, such as ciprofloxacin and ofloxacin.

(c) There is no MIC data in the '942 patent relative to moxifloxacin.

Thus, when I read the '942 patent, I would not consider that one of the compounds disclosed in this document is particularly useful for treating ophthalmic infections, nor would I assume that moxifloxacin has better properties than the other compounds disclosed in this document. In any event, there is no data in the '942 patent from which the efficacy of moxifloxacin in the treatment of ophthalmic infections via topical application to the eye might be predicted.

10. Although the '942 patent does not provide any insight relative to the possible efficacy of moxifloxacin in the treatment of ophthalmic infections, an article by J.M. Woodcock et al., published in the scientific journal Antimicrobial Agents and Chemotherapy (American Society of Microbiology) in January of

1997, did provide relevant information. A copy of the Woodcock et al. article is attached as Appendix B.

11. As indicated in the abstract on page 1 of the Woodcock et al. article, the authors found that moxifloxacin, which is referred to in the article by means of the code number "BAY 12-8039", was less active against *Pseudomonas aeruginosa* than ciprofloxacin. In fact, the data presented in Table 1 on page 102 of the article indicate that moxifloxacin is 2 to 8 fold less active against *Pseudomonas aeruginosa* than ciprofloxacin, depending on the MIC level tested, i.e., 50% or 90%. (There was an 8 fold difference at the 50% value, i.e., 2 μg/ml for moxifloxacin versus 0.25 μg/ml for ciprofloxacin, and a 2 fold difference at the 90% value, i.e., 8 μg/ml versus 4 μg/ml).

12. Based on the data presented in the Woodcock et al. article, a scientist familiar with the requirements for ophthalmic anti-infective products could not have predicted that moxifloxacin would be superior to ciprofloxacin and other fluoroquinolones as an ocular anti-infective agent, because it exhibited relatively poor activity against one of the bacterium that is of greatest concern relative to sight-threatening ophthalmic infections. In fact, I was aware of the findings of Woodcock, et al. regarding the poor *in vitro* activity of moxifloxacin against *Pseudomonas aeruginosa,* compared to ciprofloxacin, and this inferiority was a major concern. Surprisingly, however, the superior ocular penetration properties of moxifloxacin *in vivo* more than compensated for the limited *in vitro* activity of this compound against *Pseudomonas aeruginosa.*

13. Contrary to the prediction that might have been made based on the Woodcock, et al. article, Alcon's scientists discovered that the overall potency and penetration of our ophthalmic moxifloxacin compositions is, in fact, much greater than that of formulations containing ciprofloxacin, ofloxacin and other quinolones previously utilized to treat ophthalmic infections. This superiority is largely due to the ability of the compound in solution to penetrate the cornea, which is clearly not mentioned or implied in the '942 patent.

14. In one of Alcon's initial evaluations of ophthalmic compositions containing moxifloxacin, we utilized compositions containing moxifloxacin at concentrations of 0.2, 0.3 and 0.5% (wt. %) to evaluate the efficacy of ocular moxifloxacin compositions in the treatment of keratitis infections attributable to *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Keratitis is an infection involving the intrastromal layer of the corneal tissues. In order to treat this condition effectively, the anti-infective agents utilized must be capable of penetrating into the cornea. An animal model has been developed to evaluate anti-infective agents relative to this objective. See Hobden, et al., "*Pseudomonas aeruginosa* keratitis in leukopenic rabbits", Current Eye Research, volume 12, pages 461-467 (1993); a copy is attached as Appendix C. In early 1999, a study based on this model was conducted by one of the authors of the above-identified article, Richard O'Callaghan. The results of the study are provided in the attached Technical

Report (Appendix D). The results of this study confirmed that ophthalmic compositions containing moxifloxacin at concentrations of 0.2, 0.3 and 0.5%, respectively, produce a level of drug in the corneal tissues that is efficacious. Moreover, it was determined that although the *in vitro* activity of moxifloxacin against *Pseudomonas aeruginosa* is 8 fold less than the activity of ciprofloxacin, the 0.3% moxifloxacin solution utilized in this evaluation produced results equivalent to those seen in our prior testing of a 0.3% ciprofloxacin solution (i.e., CILOXAN® Solution) in the same model. The ability of the 0.3% moxifloxacin solution to match the results obtained with a ciprofloxacin solution, despite the inferior *in vitro* activity of moxifloxacin against *Pseudomonas aeruginosa*, is the result of the superior corneal penetration achieved with the moxifloxacin compositions. This evidence of superior ocular penetration was confirmed in subsequent studies conducted by various investigators. Those studies are discussed below.

15. The pharmacokinetics of moxifloxacin relative to several other fluoroquinolones has been extensively studied by my colleagues at Alcon, as well as other scientists and physicians. The findings of numerous scientists regarding the properties of moxifloxacin and other fluoroquinolones are discussed in a series of scientific articles published as a Special Supplement to the November, 2005 edition of Survey of Ophthalmology, International Review Journal (volume 50, supplement 1). A copy of this publication is attached as Appendix E. The article by Robertson, et al., which begins on page S32 of the publication, is specifically directed to a survey of studies relating to the pharmacokinetic properties of ophthalmic moxifloxacin formulations and other ophthalmic fluoroquinolone formulations.

16. The data presented in the attached article by Robertson, et al. (Appendix E, pages S32-S45) demonstrate the superior ocular bioavailability of moxifloxacin, relative to other fluoroquinolones. For example, the corneal permeability data presented in Table 3 (page S36) show that moxifloxacin penetrates the cornea much more readily than other fluoroquinolones. The ability of moxifloxacin to penetrate the cornea following application via an aqueous solution enables this drug to reach intraocular tissues in amounts sufficient to treat or prevent infections in those tissues. As shown in Table 4 (page S37), moxifloxacin reaches the intraocular fluid (i.e., aqueous humor) and intraocular tissues (e.g., iris-ciliary body) at much higher levels than ofloxacin. Similar results are shown in Table 6 (page S39) and Table 10 (page S43). The superior ocular bioavailability of moxifloxacin in solution is a surprising finding that is not predicted by the Petersen, et al., Cagle, et al and Bergamini, et al. publications cited by the Examiner in the Office Action.

17. The experiments described in the attached Robertson, et al. article (Appendix E, pages S-43-S45 involved testing of ophthalmic solutions containing moxifloxacin at concentrations of 0.3 and 0.5%. The broadest claim of the pending application specifies a concentration range of 0.1 to 1%. The superior

ocular penetration properties of moxifloxacin are not dependent on the use of a particular concentration within this range (e.g., 0.3% or 0.5%). Rather, as explained in greater detail below, these properties are prevalent over the entire range of 0.1 to 1%.

18. The bioavailability of drugs when administered via topical application to the eye can be evaluated by various ocular penetration models. Such models have proven to be quite reliable in simulating actual *in vivo* drug levels. One such model is described in Schoenwald, et al., "Corneal Penetration Behavior of ß-Blocking Agents I", Journal of Pharmaceutical Sciences, volume 72, no. 11, pages 1266-1272, November 1983; a copy is attached as Appendix F. This model measures the rate of diffusion of a drug across the cornea. It was utilized to evaluate the ocular penetration properties of moxifloxacin upon application of ophthalmic compositions containing this compound at concentrations of 0.1, 0.3, 0.5, 0.75 and 1.0%, respectively. An aqueous vehicle corresponding to the vehicle used in Alcon's VIGAMOX® (0.5% moxifloxacin) Ophthalmic Solution was utilized for these compositions.* The results of this experiment were as follows:

Drug Concentration	Diffusion Rate (Micrograms/ Minute)	Total Amount of Drug Penetration at 240 Minutes in Micrograms
0.1%	0.86	180
0.3%	2.16	455
0.5%	3.20	667
0.75%	4.54	930
1.0%	6.00	1,197

A graph and table showing the relative diffusion rates, as well as the total amount of moxifloxacin which penetrated the cornea following application of the respective compositions, are attached as Appendices G1 and G2, respectively. The results show that increasing the concentration of moxifloxacin increases the rate of diffusion of moxifloxacin across the cornea, as well as the amount of moxifloxacin that accumulates on the endothelial (aqueous humor) side of the cornea. As shown in Appendix G1, the relationship between moxifloxacin concentration and ocular penetration is linear over the concentration range of 0.1 to 1.0%.

19. The *in vitro* corneal penetration model discussed in paragraph 18 above has been utilized to compare the ocular penetration of ophthalmic solutions containing moxifloxacin and gatifloxacin, respectively. It was found that the corneal penetration of moxifloxacin is about 3.6 times greater than that of

* The VIGAMOX® vehicle contains boric acid, sodium chloride and water, and has a pH of 6.8.

gatifloxacin. This finding is described in the following publication: Owen, et al. "Corneal Penetration and Changes in Corneal Permeability of Moxifloxacin versus Gatifloxacin", Investigative Ophthalmology Visual Science 45: E-Abstract 4910, 2004; a copy is attached as Appendix H. The relative ocular penetration properties of moxifloxacin and gatifloxacin have also been evaluated via *in vivo* testing, as described in the following publication: Kim, et al., "Aqueous Penetration and Biological Activity of Moxifloxacin 0.5% Ophthalmic Solution and Gatifloxacin 0.3% Solution in Cataract Surgery Patients", Ophthalmology, volume 112, issue 11, pages 1992-1996, November 2005; a copy is attached as Appendix I. The *in vivo* study described in the foregoing article revealed that the aqueous humor level of moxifloxacin following topical application of a 0.5% moxifloxacin solution was 3.8 times greater than the aqueous humor level of gatifloxacin following application of a 0.3% gatifloxacin solution. Thus, in both the *in vitro* corneal penetration model and in the *in vivo* studies, moxifloxacin penetrated the cornea about 3.6 to 3.8 times better than gatifloxacin. This correlation between the results in the *in vitro* model and those seen in the *in vivo* testing demonstrates the reliability of the *in vitro* model as a tool for predicting the *in vivo* ocular penetration of fluoroquinolones, such as moxifloxacin. The *in vitro* data discussed in paragraph 18 above is therefore believed to provide a very realistic representation of the ocular penetration properties in patients that are treated with the ophthalmic compositions of our invention containing 0.1 to 1.0 % moxifloxacin.

20. In summary, the superior ocular bioavailability properties of moxifloxacin, when administered topically via the compositions of the present invention, has been demonstrated via numerous studies conducted by Alcon's scientists and others engaged in the field of ophthalmic anti-infective research. This superiority applies to both prior second generation fluoroquinolones, such as ciprofloxacin and ofloxacin, and fourth generation fluoroquinolones, such as gatifloxacin. These superior ocular bioavailability properties are not suggested in any manner by the Petersen, et al. reference or other references cited in the Office Action, nor any other prior publications of which I am aware. The properties are therefore truly unexpected. The experimental testing discussed in paragraph 18 above shows that the ocular penetration properties of moxifloxacin are linear across the entire 0.1 to 1.0 % concentrations utilized in the compositions and methods of treatment of our invention. The *in vitro* testing methodology described in paragraph 18 has been demonstrated to be a reliable model for the *in vivo* bioavailability of ophthalmic anti-infective agents. Based on these findings, as well as the numerous comparative tests of ophthalmic compositions containing moxifloxacin discussed in Appendix E, it can be reasonably concluded that the unexpected penetration obtained with our invention occurs at all concentrations across the range of 0.1 to 1.0 %.

21. The unexpected results achieved with our invention have greatly contributed to its commercial success. Alcon Laboratories, Inc. introduced a new anti-infective product based on our invention, i.e., VIGAMOX® (0.5% moxifloxacin) Ophthalmic Solution in 2003. The acceptance of VIGAMOX® in the medical community has been very rapid. This product was launched in the U.S. in 2003, and achieved total annual global sales of more than $100,000,000 in 2004. The acceptance of this product has continued to grow dramatically, with global sales in 2005 of about $146,000,000 and global sales in 2006 of about $185,000,000.

22. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Declared at: Fort Worth, Texas, USA, on this 22nd day of May, 2007.

David W. Stroman

David W. Stroman, Ph.D.

Attachments:
Appendix A
Appendix B
Appendix C
Appendix D
Appendix E
Appendix F
Appendix G1 and G2
Appendix H
Appendix I

CURRICULUM VITAE

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Education:

1962-1966	B.S., Chemistry, Southern Nazarene University, Bethany, OK
1966-1970	Ph.D., Biochemistry and Molecular Biology, University of Oklahoma Medical School, Oklahoma City, OK Thesis: Expression of the clustered arginine genes of *E. coli*
1970-1972	Postdoctoral studies, Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO
1974	4th International Training Course in Membrane Biophysics, Yale University School of Medicine, New Haven, CN

Professional Experience:

8/90 to present	Director, Anti-Infective Microbiology, Alcon Research, Ltd., Ft. Worth, TX
6/88-7/90	President, Bissendorf Biosciences Inc., Richardson, TX
6/87-6/88	Manager, Biotechnology Ventures, Phillips Petroleum Company
10/85-6/88	Vice President, Phillips 66 Biosciences Corporation

Formed and obtained financing for three "biotech" companies as a part of Phillips overall biopharmaceutical strategy.

Served on the Board of Directors for the three companies:

-Biosciences Corporation of Texas, Houston, TX
(J/V with Baylor College of Medicine)
-Bissendorf Biosciences, GmbH, Hannover, W. Germany
(J/V with Bissendorf Peptide, GmbH)
-Wadley Biosciences, Ltd., Dallas, TX
(J/V with Wadley Institutes of Molecular Medicine)

8/85-6/87	Coordinator for Biotechnology Licensing, Patent and Licensing Division, Phillips Petroleum Company
12/81-1/86	Scientific liaison between Phillips Petroleum and SIBIA (Phillips's J/V with The Salk Institute) with technical responsibility for contract research
12/84-8/85	Section Supervisor, Recombinant DNA Product Research, Biotechnology Division, Phillips Petroleum Company
7/81-12/84	Research Molecular Biologist and Group Leader, Biotechnology Division, Phillips Petroleum Company, Bartlesville, OK
9/76-12/76	Visiting Instructor, Department of Biology, Kalamazoo College, Kalamazoo, MI
12/72-7/81	Research Scientist, Infectious Diseases Research, The Upjohn Company, Kalamazoo, MI
9/70-11/72	NIH (NCI) Postdoctoral Fellow, Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO
6/67-8/70	Research Assistant, Department of Biochemistry and Molecular Biology, University of Oklahoma Medical School
9/66-5/67	Teaching Assistant, Department of Biochemistry and Molecular Biology, University of Oklahoma Medical School, Oklahoma City, OK
9/64-8/66	Research Assistant, Department of Chemistry, Southern Nazarene University
9/64-5/65 9/65-7/66	Teaching Assistant, Department of Chemistry, Southern Nazarene University, Bethany, OK

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In Vitro Activity of BAY 12-8039, a New Fluoroquinolone

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The in vitro activity of BAY 12-8039, a new fluoroquinolone, was studied in comparison with those of ciprofloxacin, trovafloxacin (CP 99,219), cefpodoxime, and amoxicillin-clavulanate against gram-negative, gram-positive, and anaerobic bacteria. Its activity against mycobacteria and chlamydia was also investigated. BAY 12-8039 was active against members of the family *Enterobacteriaceae* (MIC at which 90% of strains tested were inhibited [MIC_{90}s] ≤1 μg/ml, except for *Serratia* spp. MIC_{90} 2 μg/ml, *Neisseria* spp. (MIC_{90}s 0.015 μg/ml), *Haemophilus influenzae* (MIC_{90}, 0.03 μg/ml), and *Moraxella catarrhalis* (MIC_{90} 0.12 μg/ml), and these results were comparable to those obtained for ciprofloxacin and trovafloxacin. Against *Pseudomonas aeruginosa*, the quinolones were more active than the β-lactam agents but BAY-12-8039 was less active than ciprofloxacin. Strains of *Stenotrophomonas maltophilia* were fourfold more susceptible to BAY 12-8039 and trovafloxacin (MIC_{90}s, 2 μg/ml) than to ciprofloxacin. BAY 12-8039 was as active as trovafloxacin but more active than ciprofloxacin against *Streptococcus pneumoniae* (MIC_{90}, 0.25 μg/ml) and methicillin-susceptible *Staphylococcus aureus* (MIC_{90}s, 0.12 μg/ml). The activity of BAY 12-8039 against methicillin-resistant *S. aureus* (MIC_{90}, 2 μg/ml) was lower than that against methicillin-susceptible strains. BAY 12-8039 was active against anaerobes (MIC_{90}s ≤ 2 μg/ml), being three- to fourfold more active against *Bacteroides fragilis*, *Prevotella* spp., and *Clostridium difficile* than was ciprofloxacin. Against *Mycobacterium tuberculosis*, BAY 12-8039 exhibited activity comparable to that of rifampin (MICs ≤ 0.5 μg/ml). Against *Chlamydia trachomatis* and *Chlamydia pneumoniae* BAY 12-8039 was more active (MICs ≤ 0.12 μg/ml) than either ciprofloxacin or erythromycin and exhibited a greater lethal effect than either of these two agents. The protein binding of BAY 12-8039 was determined at 1 and 5 μg/ml as 30 and 26.4%, respectively. The presence of human serum (at 20 or 70%) had no marked effect on the in vitro activity of BAY 12-8039.

BAY 12-8039 is a new fluoroquinolone derivative with a chemical nomenclature of 1-cyclopropyl-7-[(*S,S*)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid. It shares structural similarities with other agents, namely, a cyclopropyl group at position 1 (as ciprofloxacin has), a methoxy group at position 8 (as AM1155 has) (10), and a diazabicyclo group at position 7 (as BAY y3118 has) (5). Preliminary information suggests that BAY 12-8039 has enhanced activity against gram-positive bacterial pathogens (3). In this study, the activity of BAY 12-8039 was compared with that of other fluoroquinolones and the novel naphthyridone compound trovafloxacin (CP 99,219) (2) against a wide range of pathogens.

MATERIALS AND METHODS

Antimicrobial agents. The following agents were employed: BAY 12-8039 and ciprofloxacin (Bayer AG, Wuppertal, Germany), trovafloxacin (Pfizer Limited, Groton, Conn.), cefpodoxime (Roussel Uclaf, Romainville, France), amoxicillin and clavulanic acid (SmithKline Beecham, Worthing, United Kingdom), rifampin (Sigma, Poole, United Kingdom), rifampin (Sigma, Poole, United Kingdom), and erythromycin (Lilly Products, Basingstoke, United Kingdom). All agents were prepared and stored following the manufacturer's instructions.

Susceptibility testing. A total of 684 recent clinical isolates, 11 control strains, and 10 well-characterized β-lactamase-producing strains were studied. The control strains used were *Escherichia coli* NCTC 10418 and ATCC 25922, *Pseudomonas aeruginosa* NCTC 10662 and ATCC 27853, *Staphylococcus aureus* NCTC 6571 and ATCC 29213, *Streptococcus pneumoniae* NCTC 7465 and ATCC 49619, *Haemophilus influenzae* NCTC 11931 and ATCC 49247, and *Enterococcus faecalis* ATCC 29212. Susceptibilities were determined by a standard agar plate dilution method following recommendations in reference 1. Briefly, Iso-Sensitest agar (pH 7.2; Unipath, Basingstoke, United Kingdom) was employed for aerobic bacteria, supplemented with 50 μg of 1-(4-nitrophenyl)-glycerol (BDH, Poole, United Kingdom) per ml where necessary to prevent swarming. Supplements of 5% horse blood (Bradacre Biologicals, Loughborough, United Kingdom) and 20 μg of NAD (Sigma) per ml were added to support growth of fastidious bacteria. For anaerobic bacteria, Wilkins-Chalgren agar (Unipath) supplemented with 50 μg of 1-(4-nitrophenyl)-glycerol per ml and 5% horse blood was used. All strains were tested at a final inoculum of 10^4 CFU and for a few selected strains at an increased inoculum of 10^5 CFU, using a multipoint inoculator (Denley Instruments, Billingshurst, United Kingdom). Plates were incubated at 35 to 37°C for 18 to 24 h in air; or, for fastidious bacteria, in an atmosphere enriched with 4 to 6% carbon dioxide; or, for anaerobic bacteria, in an anaerobic cabinet (Don Whitley, Shipley, United Kingdom) in an atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen.

The MIC was defined as the lowest antibiotic concentration at which no more than two colonies were observed. Amoxicillin and clavulanic acid were combined in a ratio of 2:1, and the results were recorded in terms of the amoxicillin MIC.

Mycobacterium susceptibility testing. The activity of BAY 12-8039 against mycobacteria was studied by an agar incorporation method using rifampin as a comparative agent. Recent clinical isolates of *Mycobacterium tuberculosis* (three resistant to one or more of the commonly used antimycobacterial agents and one susceptible strain) were studied. For both antibiotics a concentration range of 0.015 to 128 μg/ml (doubling dilutions up or down from 1 μg/ml) incorporated into Middlebrook 7H10 medium (Difco, Detroit, Mich.), containing 10% Middlebrook oleic acid-albumin-dextrose-catalase enrichment as a supplement, was used. Plates were incubated at 37°C in 5 to 10% carbon dioxide for 21 days. The lowest concentration of antibiotic that inhibited more than 99% of the bacterial population was considered to be the MIC (8).

Chlamydia susceptibility testing. The activity of BAY 12-8039 against one strain of *Chlamydia pneumoniae* and 3 strains of *Chlamydia trachomatis* was investigated in comparison with those of ciprofloxacin and erythromycin. The method employed was an adaptation of that of Webberley et al. (11). The MIC was taken as the lowest concentration to inhibit the development of inclusion bodies, and the minimum lethal concentration (MLC) was defined by the absence of inclusion bodies after a further 48-h incubation in drug-free medium.

Serum effect. The effect of human serum on the MIC and minimum bactericidal concentration (MBC) of BAY 12-8039 was determined for two strains each of *Streptococcus pyogenes*, *S. pneumoniae*, methicillin-sensitive *S. aureus* (MSSA), *Moraxella catarrhalis*, *E. coli*, and *Klebsiella pneumoniae*. A microdilution method was employed using Iso-Sensitest broth (Unipath) containing 20 or 70% human serum (Bradacre Biologicals) and supplemented for fastidious bacteria with 5% lysed horse blood and 20 μg of NAD per ml. Concentration ranges (doubling dilutions up or down from 1 μg/ml) of BAY 12-8039 were 0.008 to 8 μg/ml or 0.03 to 32 μg/ml (for fastidious bacteria). A final inoculum of 10^5 CFU/ml was used. Following incubation at 35 to 37°C in air or 4 to 6% carbon dioxide (for fastidious bacteria), 50 μl of broth culture was subcultured onto appropriate antibiotic-free medium for MBC determinations. The MIC was defined as the

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TABLE 1. The in vitro activity of BAY 12-8039 in comparison with those of other antimicrobial agents

Organism (no.)	Antibiotic	MIC (μg/ml)*		
		50%	90%	Range
E. coli (39)	BAY 12-8039	0.06	1	0.03–32
	Trovafloxacin	0.06	1	0.015–>128
	Ciprofloxacin	0.015	0.5	0.008–64
	Cefpodoxime	0.25	4	0.12–>128
	Amoxicillin-clavulanate	2	16	0.5–32
Klebsiella spp. (30)	BAY 12-8039	0.12	0.5	0.06–4
	Trovafloxacin	0.12	0.5	0.06–8
	Ciprofloxacin	0.03	0.25	0.015–4
	Cefpodoxime	0.25	8	0.12–64
	Amoxicillin-clavulanate	4	8	1–32
P. mirabilis (30)	BAY 12-8039	0.25	0.25	0.12–0.5
	Trovafloxacin	0.25	0.25	0.12–0.5
	Ciprofloxacin	0.03	0.03	0.008–0.03
	Cefpodoxime	0.06	0.06	0.03–0.06
	Amoxicillin-clavulanate	0.5	4	0.25–8
P. vulgaris (15)	BAY 12-8039	0.25	0.25	0.06–0.5
	Trovafloxacin	0.25	0.5	0.06–1
	Ciprofloxacin	0.03	0.03	0.008–0.03
	Cefpodoxime	0.12	0.5	0.03–0.5
	Amoxicillin-clavulanate	2	8	0.5–8
M. morganii (15)	BAY 12-8039	0.12	0.25	0.03–0.25
	Trovafloxacin	0.25	0.5	0.06–1
	Ciprofloxacin	0.008	0.015	0.004–0.015
	Cefpodoxime	0.12	4	0.015–16
	Amoxicillin-clavulanate	64	64	16–128
Serratia spp. (20) [*S. marcescens* (15); *S. liquefaciens* (4)]	BAY 12-8039	0.5	2	0.03–16
	Trovafloxacin	0.5	4	0.06–64
	Ciprofloxacin	0.12	1	0.015–16
	Cefpodoxime	4	64	1–>128
	Amoxicillin-clavulanate	64	128	8–>128
Acinetobacter spp. (15) [*A. baumannii* (11); *A. haemolyticus* (3)]	BAY 12-8039	0.06	2	0.008–16
	Trovafloxacin	0.03	1	0.004–16
	Ciprofloxacin	0.25	8	0.015–128
	Cefpodoxime	16	>128	1–>128
	Amoxicillin-clavulanate	8	64	2–>128
P. aeruginosa (15)	BAY 12-8039	2	8	0.12–64
	Trovafloxacin	0.5	8	0.03–128
	Ciprofloxacin	0.25	4	0.015–32
	Cefpodoxime	>128	>128	128–>128
	Amoxicillin-clavulanate	128	>128	32–>128
S. maltophilia (13)	BAY 12-8039	0.5	2	0.06–2
	Trovafloxacin	0.5	2	0.12–8
	Ciprofloxacin	2	8	0.25–16
	Cefpodoxime	>128	>128	64–>128
	Amoxicillin-clavulanate	128	>128	64–>128
Enterobacter spp. (5)	BAY 12-8039			0.12
	Trovafloxacin			0.06–0.12
	Ciprofloxacin			0.015–0.03
	Cefpodoxime			1–>128
	Amoxicillin-clavulanate			4–128
Citrobacter spp. (5) [*C. diversus* (3); *C. freundii* (2)]	BAY 12-8039			0.03–0.25
	Trovafloxacin			0.03–0.25
	Ciprofloxacin			0.008–0.06
	Cefpodoxime			0.25–2
	Amoxicillin-clavulanate			1–32
Salmonella spp. (5)	BAY 12-8039			0.06–1
	Trovafloxacin			0.06–1
	Ciprofloxacin			0.03–0.25
	Cefpodoxime			0.5–2
	Amoxicillin-clavulanate			0.5–16
Shigella spp. (5)	BAY 12-8039			0.03–0.06
	Trovafloxacin			0.015–0.06
	Ciprofloxacin			0.015
	Cefpodoxime			0.25–0.5
	Amoxicillin-clavulanate			1–8
Providencia spp. (15) [*P. stuartii* (11); *P. rettgeri* (2); *P. alcalifaciens* (2)]	BAY 12-8039	0.25	0.5	0.06–1
	Trovafloxacin	0.12	0.25	0.06–1

Continued on following page

TABLE 1—*Continued*

Organism (no.)	Antibiotic	MIC (μg/ml)*		
		50%	90%	Range
	Ciprofloxacin	0.03	0.25	0.015–0.25
	Cefpodoxime	0.03	1	0.03–16
	Amoxicillin-clavulanate	128	128	2–128
MSSA (54)	BAY 12-8039	0.06	0.12	0.03–0.12
	Trovafloxacin	0.03	0.06	0.015–0.12
	Ciprofloxacin	0.5	1	0.12–2
	Cefpodoxime	2	4	0.5–4
	Amoxicillin-clavulanate	0.25	0.5	0.12–1
MRSA (20)	BAY 12-8039	2	2	2
	Trovafloxacin	2	2	2
	Ciprofloxacin	128	128	32–128
	Cefpodoxime	>128	>128	128–>128
	Amoxicillin-clavulanate	16	16	16–32
S. epidermidis (29)	BAY 12-8039	0.06	2	0.03–2
	Trovafloxacin	0.03	4	0.15–4
	Ciprofloxacin	0.25	8	0.12–32
	Cefpodoxime	1	16	0.5–>128
	Amoxicillin-clavulanate	0.12	2	0.12–64
S. saprophyticus (30)	BAY 12-8039	0.12	0.25	0.12–0.25
	Trovafloxacin	0.06	0.12	0.06–0.12
	Ciprofloxacin	0.5	0.5	0.25–0.5
	Cefpodoxime	4	8	2–8
	Amoxicillin-clavulanate	0.25	0.5	0.12–0.5
S. pneumoniae (32)	BAY 12-8039	0.12	0.25	0.06–8
	Trovafloxacin	0.12	0.25	0.06–8
	Ciprofloxacin	1	16	0.5–128
	Cefpodoxime	0.5	4	0.03–8
	Amoxicillin-clavulanate	0.12	1	0.015–1
S. milleri (30)	BAY 12-8039	0.12	0.25	0.06–0.25
	Trovafloxacin	0.12	0.25	0.06–0.25
	Ciprofloxacin	0.5	1	0.5–8
	Cefpodoxime	0.25	0.5	0.03–32
	Amoxicillin-clavulanate	0.12	0.12	0.03–0.25
Group A streptococci (20)	BAY 12-8039	0.25	0.25	0.06–0.25
	Trovafloxacin	0.12	0.25	0.06–0.25
	Ciprofloxacin	0.5	1	0.25–1
	Cefpodoxime	0.015	0.015	0.015
	Amoxicillin-clavulanate	0.015	0.015	0.015
Group B streptococci (20)	BAY 12-8039	0.25	0.25	0.06–0.5
	Trovafloxacin	0.25	0.25	0.12–0.5
	Ciprofloxacin	1	1	0.5–2
	Cefpodoxime	0.06	0.06	0.03–0.06
	Amoxicillin-clavulanate	0.06	0.06	0.06
E. faecalis (30)	BAY 12-8039	0.25	0.5	0.12–4
	Trovafloxacin	0.25	0.5	0.12–8
	Ciprofloxacin	2	2	1–32
	Cefpodoxime	8	>128	1–>128
	Amoxicillin-clavulanate	0.5	0.5	0.12–16
E. faecium (20)	BAY 12-8039	2	2	0.25–4
	Trovafloxacin	0.5	2	0.25–8
	Ciprofloxacin	2	4	1–8
	Cefpodoxime	>128	>128	0.5–>128
	Amoxicillin-clavulanate	4	16	0.12–16
H. influenzae (36)	BAY 12-8039	0.03	0.03	0.015–0.06
	Trovafloxacin	0.008	0.015	0.004–0.03
	Ciprofloxacin	0.015	0.015	0.008–0.015
	Cefpodoxime	0.06	0.12	0.03–0.5
	Amoxicillin-clavulanate	0.5	2	0.25–4
M. catarrhalis (35)	BAY 12-8039	0.06	0.12	0.06–0.12
	Trovafloxacin	0.03	0.03	0.008–0.06
	Ciprofloxacin	0.06	0.06	0.03–0.06
	Cefpodoxime	0.5	1	0.12–16
	Amoxicillin-clavulanate	0.12	0.25	0.015–1
N. gonorrhoeae (34)	BAY 12-8039	0.008	0.015	0.004–0.12
	Trovafloxacin	0.004	0.008	0.002–0.03
	Ciprofloxacin	0.004	0.004	0.001–0.12

Continued on following page

TABLE 1—*Continued*

Organism (no.)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
	Cefpodoxime	0.008	0.015	0.002–0.03
	Amoxicillin-clavulanate	0.25	1	0.05–1
N. meningitidis (10)	BAY 12-8039	0.008	0.015	0.004–0.015
	Trovafloxacin	0.004	0.008	0.004–0.008
	Ciprofloxacin	0.008	0.008	0.004–0.008
	Cefpodoxime	0.004	0.004	0.002–0.008
	Amoxicillin-clavulanate	0.06	0.12	0.03–0.12
Peptostreptococcus spp. (20)	BAY 12-8039	0.12	1	0.05–1
	Trovafloxacin	0.5	1	0.05–2
	Ciprofloxacin	1	2	0.12–2
	Cefpodoxime	1	4	0.25–64
	Amoxicillin-clavulanate	0.06	0.25	0.05–16
B. fragilis (25)	BAY 12-8039	0.25	0.25	0.12–1
	Trovafloxacin	1	1	0.5–1
	Ciprofloxacin	2	4	1–4
	Cefpodoxime	64	>128	2–>128
	Amoxicillin-clavulanate	0.5	2	0.5–4
Prevotella spp. (3)	BAY 12-8039			0.12–0.25
	Trovafloxacin			0.25–1
	Ciprofloxacin			2
	Cefpodoxime			2–>128
	Amoxicillin-clavulanate			2
Clostridium perfringens (10)	BAY 12-8039	0.5	0.5	0.25–1
	Trovafloxacin	0.5	1	0.5–1
	Ciprofloxacin	0.5	0.5	0.25–0.5
	Cefpodoxime	16	32	1–32
	Amoxicillin-clavulanate	0.25	0.25	0.06–0.25
C. difficile (10)	BAY 12-8039	1	2	1–2
	Trovafloxacin	2	2	2
	Ciprofloxacin	16	16	16
	Cefpodoxime	>128	>128	128–>128
	Amoxicillin-clavulanate	0.5	1	0.25–2

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

lowest antibiotic concentration at which there was no visible growth, and the MBC was defined as the lowest antibiotic concentration to reduce growth to five colonies or fewer (equivalent to 99.9% lethality) (7a).

Protein binding determinations. The protein binding of BAY 12-8039 at two concentrations (1 and 5 μg/ml) in pooled human serum (Bradsure Biologicals) was investigated. The method employed Centrifree ultrafiltration units (Amicon, Stonehouse, United Kingdom). Ultrafiltrates were assayed against BAY 12-8039 phosphate buffer (pH 7) calibrators by a microbiological plate assay.

RESULTS

The activity of BAY 12-8039 against members of the family *Enterobacteriaceae* (MIC at which 90% of strains tested were inhibited [MIC_{90}] ≤ 1 μg/ml, and for *Serratia* spp. MIC_{90} = 2 μg/ml) was similar to that observed for trovafloxacin (Table 1). Both these agents were generally one-half as active as ciprofloxacin, except against *Proteus mirabilis*, *Proteus vulgaris*, *Morganella morganii*, *Enterobacter* spp., and *Citrobacter* spp., where ciprofloxacin was 8 to 16 times more active. In general, the quinolones were more active than either of the β-lactam agents against members of the *Enterobacteriaceae*. BAY 12-8039 was equally active against β-lactamase-producing and -nonproducing strains of *E. coli*.

BAY 12-8039 was shown to be more active against *Acinetobacter* spp. (MIC_{90}, 2 μg/ml) than ciprofloxacin (MIC_{90}, 8 μg/ml). Against *P. aeruginosa* and *Stenotrophomonas maltophilia* the quinolones, including BAY 12-8039, were more active (MIC_{90} ≤ 8 μg/ml) than the β-lactam agents (MIC_{90}s > 128 μg/ml). Both BAY 12-8039 and trovafloxacin were more active against *S. maltophilia* (MIC_{90}s, 2 μg/ml) than ciprofloxacin (MIC_{90}s, 8 μg/ml).

BAY 12-8039 exhibited activity against *Staphylococcus saprophyticus* (MIC_{90}, 0.25 μg/ml) and *Staphylococcus epidermidis* (MIC_{90}, 2 μg/ml), the MIC_{90}s of ciprofloxacin being 0.5 and 8 μg/ml, respectively. The activity of BAY 12-8039 against MSSA (MIC_{90}, 0.12 μg/ml) was similar to that of trovafloxacin (MIC_{90}, 0.06 μg/ml) but greater than that of ciprofloxacin (MIC_{90}, 1 μg/ml). BAY 12-8039 was less active against methicillin-resistant *S. aureus* (MRSA) (MIC_{90}, 2 μg/ml) than against methicillin-susceptible strains (MIC_{90}, 0.12 μg/ml). However, it was more active than ciprofloxacin (MIC_{90}, 128 μg/ml), cefpodoxime (MIC_{90}, >128 μg/ml), and amoxicillin-clavulanate (16 μg/ml) against the MRSA.

TABLE 2. In vitro activity of BAY 12-8039 in comparison with rifampin against *M. tuberculosis*

Strain	Resistance pattern to commonly used antimycobacterial agents	MIC (μg/ml) of:	
		BAY 12-8039	Rifampin
1	Fully sensitive	0.5	0.25
2	Isoniazid and streptomycin resistant	0.25	0.25
3	Isoniazid and rifampin resistant	0.12	ND[a]
4	Streptomycin resistant	0.25	0.5

[a] ND, not determined.

TABLE 3. MIC and MLC of BAY 12-8039 and comparator agents for *C. trachomatis* and *C. pneumoniae*[a]

Strain	BAY 12-8039		Ciprofloxacin		Erythromycin	
	MIC	MLC	MIC	MLC	MIC	MLC
C. trachomatis 6/96	0.06	0.12	2.0	2.0	0.25	2.0
C. trachomatis 7/96	0.12	0.12	2.0	2.0	0.5	4.0
C. trachomatis 8/96	0.06	0.12	1.0	2.0	0.5	4.0
C. pneumoniae TW183	0.06	0.06	2.0	2.0	0.25	0.5

[a] Values are given in micrograms per milliliter.

BAY 12-8039 exhibited activity against *Streptococcus milleri* and group A and group B streptococci (MIC_{90}s, 0.25 μg/ml), and this was comparable to that of trovafloxacin. The activity of BAY 12-8039 against *S. pneumoniae* (MIC_{90}, 0.25 μg/ml) was also similar to that of trovafloxacin but was considerably greater than that of ciprofloxacin (MIC_{90}, 16 μg/ml). A strain inhibited by 16 μg of ciprofloxacin per ml was inhibited by 0.12 and 0.25 μg of BAY 12-8039 and trovafloxacin per ml, respectively. BAY 12-8039 was also shown to be active against *E. faecalis* (MIC_{90}, 0.5 μg/ml) and *Enterococcus faecium* (MIC_{90}, 2 μg/ml).

BAY 12-8039, in common with the other quinolones, was highly active against *Neisseria gonorrhoeae* and *Neisseria meningitidis* (MIC_{90}, 0.015 μg/ml), *H. influenzae* (MIC_{90}, 0.03 μg/ml), and *M. catarrhalis* (MIC_{90}, 0.12 μg/ml).

BAY 12-8039 was found to be active against all the strains of anaerobic bacteria studied (MIC_{90}s ≤ 2 μg/ml). BAY 12-8039 was three or fourfold more active against *Bacteroides fragilis*, *Prevotella* spp., and *Clostridium difficile* than ciprofloxacin.

BAY 12-8039 exhibited an activity comparable to that of rifampin for all strains of *M. tuberculosis* (Table 2).

Against both *C. trachomatis* and *C. pneumoniae* (Table 3) BAY 12-8039 was shown to be more active (MICs of 0.06 to 0.12 μg/ml) than either ciprofloxacin (MICs of 1 to 2 μg/ml) or erythromycin (MICs of 0.25 to 0.5 μg/ml). BAY 12-8039 exhibited a high lethal effect against both *C. trachomatis* and *C. pneumoniae*, with the MLCs being equal to, or within one dilutional step of, the MICs.

An increase in inoculum size from 10^4 to 10^6 did not affect the MICs for the *E. coli* strains studied (data not shown). For *K. pneumoniae*, however, one strain was affected, and in this case the MIC increased fourfold. The majority of *P. mirabilis* strains tested at an increased inoculum showed a twofold increase in MIC. For *S. marcescens*, two of five strains showed a threefold increase in MIC.

The presence of human serum had no marked effect on the MICs or MBCs determined for BAY 12-8039 at either 20 or 70% (Table 4), with the exception of one strain of group A streptococci for which the MBC was 0.25 μg/ml in the absence of serum and 1 μg/ml in the presence of 70% serum. The protein binding of BAY 12-8039 was determined at 1 and 5 μg/ml as 30 and 26.4%, respectively.

DISCUSSION

The results presented here generally agree with preliminary information on BAY 12-8039 which indicates improved in vitro activity against gram-positive bacteria (3). In this study, BAY 12-8039 was found to be more active than ciprofloxacin against *S. pneumoniae*, MSSA, and MRSA. In addition, the activity of BAY 12-8039 equalled that of trovafloxacin, which has previously been shown to possess improved activity against gram-positive bacteria (2, 4). It should be noted that BAY 12-8039 was less active against MRSA than against MSSA. The strains of MRSA used in this study were recent clinical isolates, and it is therefore likely that some were ciprofloxacin-resistant epidemic MRSA (6). In the clinical situation resistance to ciprofloxacin by MRSA appears to be rapidly acquired (7, 9), and it is possible that the mechanism(s) of resistance to ciprofloxacin also applies to BAY 12-8039.

In common with other fluoroquinolones, BAY 12-8039 exhibited activity against the *Enterobacteriaceae*. Against *Acinetobacter* spp. BAY 12-8039 was shown to be more active than ciprofloxacin. In addition, BAY 12-8039 was generally found to have improved activity compared to that of ciprofloxacin against anaerobic bacteria.

BAY 12-8039 was shown to be active against common respiratory pathogens, such as *M. catarrhalis* and *H. influenzae*. Against *M. tuberculosis* BAY 12-8039 was found to be as active as rifampin. This activity is similar to that of ciprofloxacin and improved compared to that of trovafloxacin (2). BAY 12-8039 was shown to be slightly more active against *Mycobacterium avium-Mycobacterium intracellulare* compared to rifampin. Against strains of *Chlamydia* spp. BAY 12-8039 was found to be more active than either erythromycin or ciprofloxacin.

The protein binding of BAY 12-8039 was similar to that of many other fluoroquinolones (<50%) but less than that of trovafloxacin, which we found to be approximately 85% em-

TABLE 4. Effect of human serum on the in vitro activity of BAY 12-8039

Organism type[a]	Agar MIC (μg/ml)	Broth MIC (μg/ml)	MBC (μg/ml)	20% Human serum		70% Human serum	
				MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
Group A streptococci	0.25	0.25	0.25	0.25	0.25	0.5	1
	0.25	0.12	0.25	0.25	0.25	0.25	0.25
S. pneumoniae	0.12	0.25	0.25	0.12	0.12	0.12	0.5
	0.25	0.25	0.25	0.25	0.25	0.25	0.5
M. catarrhalis	0.12	0.06	0.12	0.03	0.12	ND[b]	0.25
	0.12	0.06	0.12	ND	0.12	0.03	0.25
S. aureus	0.03	0.03	0.06	0.06	0.12	0.06	0.12
	0.06	0.03	0.06	0.06	0.12	0.06	0.12
E. coli	0.06	0.03	0.06	0.03	0.03	0.03	0.03
	0.03	0.03	0.03	0.015	0.03	0.03	0.03
K. pneumoniae	0.06	0.06	0.12	0.12	0.12	0.12	0.12
	0.12	0.06	0.06	0.03	0.06	0.03	0.06

[a] Two strains of each organism were studied.
[b] ND, not determined.

ploying similar methodologies (2). The presence of serum had, as expected, little or no effect upon the in vitro antimicrobial activity of the new compound.

BAY 12-8039 has a broad spectrum of activity which includes gram-negative and gram-positive bacteria, *Chlamydia* spp., *M. tuberculosis*, and anaerobes and therefore has considerable clinical potential in a wide range of infections.

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Pseudomonas aeruginosa keratitis in leukopenic rabbits

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ABSTRACT

To study the role of the host inflammatory response in *Pseudomonas aeruginosa* keratitis, rabbits were made leukopenic with intravenous injections of cyclophosphamide and dexamethasone. Twenty-four hr later, keratitis was initiated in all rabbits with an intrastromal injection of 1,000 log phase *P. aeruginosa* strain 27853. Slit lamp examination of eyes showed that leukopenic rabbits had significantly less ($P<0.0001$) ocular pathology at 16, 22, and 27 hr postinfection. The number of viable bacteria recovered from corneas of leukopenic rabbits was the same as the number recovered from nonleukopenic rabbits ($P=0.95$). These results suggest that the host inflammatory response significantly contributes to the overall ocular pathology associated with *P. aeruginosa* keratitis, but does not influence the survival of the infecting organism in the cornea at the height of the infection.

INTRODUCTION

Pseudomonas aeruginosa causes the most severe form of bacterial keratitis (1,2). Infections of the cornea with this organism are characterized by a rapid liquefactive necrosis of the stroma (3), which can lead to corneal perforation within 24 hr (2).

The pathogenesis of *P. aeruginosa* keratitis appears to involve both bacterial (4-6) and host (7-9) constituents. Bacterial factors reported to be important for ocular virulence are elastase, alkaline protease, and exotoxin A (6,10). Host-derived products which could damage the cornea are thought to be associated with polymorphonuclear leukocytes (PMN) chemotactically attracted to the site of infection (11-15).

The exact role bacterial and host factors play in the destruction of the cornea is unknown. To determine the role of the host inflammatory response in *P. aeruginosa* keratitis, rabbits in this study were made leukopenic prior to being intrastromally infected with viable *P. aeruginosa*. The development of ocular pathology in these rabbits was recorded by slit lamp examination (SLE) at 16, 22, and 27 hr postinfection (PI). The effects of leukopenia on the number of PMN infiltrating the infected cornea, as well as on the number of viable bacteria present in corneal tissue, were determined 27 hr PI.

MATERIALS AND METHODS

Induction of leukopenia

All animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research. New Zealand white rabbits (3.0±0.1 kg) were made leukopenic with injections of cyclophosphamide (75 mg/kg) and dexamethasone (4 mg/kg) as described by Stroop and Schaefer (16). Rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (20 mg/kg; Ketaset®, 100 mg/ml, Aveco Co., Inc., Fort Dodge, IA) and xylazine hydrochloride (10 mg/kg; Rompun®, 100 mg/ml, Miles Laboratories,

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Shawnee, KS). A 20 mg/ml filter-sterilized solution of cyclophosphamide monohydrate (Sigma Chemicals, St. Louis, MO) prepared in phosphate buffered saline (PBS, pH 7.4) was then injected into the marginal ear vein. Control rabbits were injected with PBS (3 ml/kg). Twenty-four hours later, dexamethasone sodium phosphate (4 mg/ml; The Butler Co., Columbus, MO) or PBS (1 ml/kg) was intravenously administered. White blood cell (WBC) counts were determined in each rabbit from blood drawn from the marginal ear vein using a Unipette kit (Becton-Dickinson, Rutherford, NJ) for manual WBC determinations prior to each injection, at the initiation of infection, and at the time of sacrifice. To differentiate the WBC types at the time of sacrifice (27 hr PI), blood smears were prepared and stained with Wright's Stain (Leukostat®, Fisher Diagnostics, Orangeburg, NY).

Experimental *Pseudomonas aeruginosa* keratitis

P. aeruginosa keratitis was initiated at 24 hr after the injection of dexamethasone in leukopenic and nonleukopenic rabbits (3 rabbits, 6 eyes per group). The procedure for initiating keratitis has been described (17). Briefly, rabbits were anesthetized with ketamine and xylazine as described above and corneas were anesthetized with 0.5% proparacaine hydrochloride (Ophthaine®, E.R. Squibb & Sons, Inc., New Brunswick, NJ). An aliquot of 10 µl of tryptic soy broth (Difco Laboratories, Detroit, MI) containing approximately 1,000 logarithmically grown *P. aeruginosa* ATCC 27853 was then injected into the stroma. *P. aeruginosa* 27853 establishes reproducible keratitis in rabbits (18-22) and guinea pigs (23,24).

Evaluation of ocular inflammation

Slit lamp examination and estimations of numbers of PMN infiltrating into corneal tissue were used to determine the effect of leukopenia on the inflammatory response resulting from the pseudomonal corneal infection.

To assess inflammatory changes in the conjunctiva, anterior chamber, and the cornea, eyes were examined with a Topcon SL-5D slit lamp biomicroscope (Kogaku Kikai K.K., Tokyo, Japan) at 16, 22, and 27 hr PI. All examinations were conducted independently in a masked fashion by three observers. The scoring system used has been previously described (22,25). Briefly, scores of 0.00 (absent) to +4.00 (severe) in 0.25 increments were assigned to seven parameters: conjunctival injection, conjunctival chemosis, iritis (cell and flare), fibrin in anterior chamber, hypopyon, stromal infiltrate, and stromal edema. Scores from each of the parameters were summed to provide a single value that represented the degree of change observed.

The numbers of PMN in corneal tissue at 27 hr PI was determined by quantitating myeloperoxidase (MPO) activity in an assay similar to that described by Williams et al. (26). Assays were conducted in 96-well microtiter plates (Costar; Cambridge, MA) and samples were run in triplicate. Rabbits were sacrificed with an overdose of pentobarbital sodium (The Butler Co.) and corneas were aseptically removed as previously described (17). Corneas were homogenized as described below for quantitation of viable *P. aeruginosa* per cornea. Aliquots of 0.1 ml of homogenate were removed for bacterial enumeration before hexadecyltrimethylammonium bromide (CTAB, Sigma) was added to a final concentration of 0.5%. The final volume of the homogenate was 3.0 ml. The mixture was further homogenized on ice for 30 sec. Tissue debris was removed from the

homogenate by centrifugation at 40,000 x g for 15 min at 4°C. A 6.9 µl aliquot of the resulting supernatant was then mixed with 200 µl of potassium phosphate buffer (50 mM, pH 6.0) containing o-dianisidine dihydrochloride (16.7 mg/100 ml, Sigma) and hydrogen peroxide (0.0005%). Optical density at 450 nm was measured every 2 min with a Dynatech MR500 microtiter plate reader (Dynatech Laboratories, Chantilly, VA) for 10-15 min at room temperature. Calculations of MPO activity were performed as described by Williams et al. (26). One unit of MPO activity is equivalent to approximately 5 logs of PMN. The lowest detectable MPO activity was 0.01 units which is equivalent to approximately 3 logs of PMN. For corneas with less than 0.01 units, a value of 0 PMN per cornea was used to calculate the average number of PMN per group. PMN determinations are expressed as the log base 10 number of PMN per cornea.

Quantitation of viable *P. aeruginosa* per cornea

Corneas surgically removed at the corneoscleral limbus were minced, placed into a sterile tube containing 3.0 ml sterile PBS (pH 7.4), and homogenized on ice with an Ultra-Turrax® Tissuemizer (Tekmar Co., Cincinnati, OH). Homogenates were serially diluted (1:10) to a dilution factor of 10^{-6}. Aliquots (0.1 ml) of each dilution (including the undiluted sample) were plated on tryptic soy agar plates (Difco Laboratories, Detroit, MI) and incubated for 24-48 hr at 37°C.

Statistical analysis of data

Statistical analysis was carried out using the Statistical Analysis System (SAS) software program (27) for personal computers. For colony forming units and log number of PMN, an analysis of variance was performed and, where a significant analysis of variance was found, t tests between the least square means from each treatment group were performed. For SLE scores, nonparametric one-way analysis of variance (Kruskal-Wallis test) was used. For comparison among groups in this analysis, Wilcoxon scores were used. A probability value of less than 0.05 was considered significant.

RESULTS

The decrease in circulating WBC after treatment with cyclophosphamide and dexamethasone is shown in Figure 1. The number of circulating WBC in drug-treated rabbits compared to control rabbits decreased 40% by day 2 (24 hours after dexamethasone injection) and even further to 80% by day 3. Peripheral blood smears showed a corresponding 50% decrease in circulating PMN in the leukopenic rabbits, compared to the nonleukopenic rabbits, at the time of sacrifice (day 3).

The infected, leukopenic rabbits demonstrated significantly less ocular pathology at 16, 22, and 27 hr ($P<0.0001$), compared to the nonleukopenic animals (Table 1). As in the peripheral circulation, the corneas from infected leukopenic rabbits had significantly fewer PMN than the corneas from nonleukopenic rabbits ($P<0.02$) (Table 2). The numbers of viable bacteria, however, were not significantly different between the two groups ($P=0.30$) (Table 2).

DISCUSSION

Infection of mice (28), guinea pigs (29), and rabbits (30) has shown that the host inflammatory response to *P. aeruginosa* keratitis consists almost entirely of infiltrating PMN. These cells migrate through the corneal stroma from limbal blood vessels (31) to the site of infection in response to chemotactic

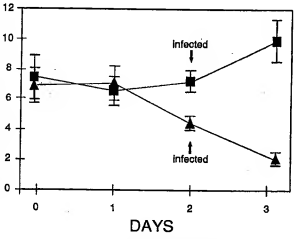

Figure 1: Induction of leukopenia in rabbits. Rabbits were made leukopenic with intravenous injections of cyclophosphamide (75 mg/kg) and dexamethasone (4 mg/kg). A sterile 20 mg/ml solution of cyclophosphamide monohydrate prepared in phosphate buffered saline (pH 7.4) was injected into the marginal ear vein on day 0. Twenty-four hr later (day 1), an injection of dexamethasone sodium phosphate (4 mg/ml) was administered intravenously. Control rabbits were injected each day with the appropriate volume of saline. The total white blood cell counts were determined in blood samples drawn from the marginal ear vein prior to the injection of cyclophosphamide (triangle) or saline (square) on day 0, and for the next 3 consecutive days. Rabbits were leukopenic 24 hr after the injection of dexamethasone (day 2) at which time bacterial keratitis was initiated. Values are means ± SEM of 3 rabbits.

stimuli originating from both the host (32,33) and the micro-organism (34). The oxidative burst in PMN and the subsequent release of free radicals and proteases (serine protease, elastase, collagenase, and gelatinase) cause extensive damage to the corneal stroma (11-15).

In 1979, Chusid and Davis (35) rendered guinea pigs neutropenic with whole body X-irradiation before intrastromally injecting an overnight culture of a strain of *P. aeruginosa* obtained from a human corneal ulcer. They reported that corneas of neutropenic guinea pigs contained one-third as many PMN and one hundred times more bacteria than corneas of non-neutropenic guinea pigs. In our study, we noted significantly fewer PMN in the corneas of leukopenic animals compared to the corneas of nonleukopenic animals. However, despite containing 2.5 logs fewer PMN, corneas of leukopenic rabbits had the same number of bacteria as the corneas of nonleukopenic rabbits. In contrast to the guinea pig model, in our rabbit model PMN were apparently unable to contain the rapid growth of bacteria.

In our study, eyes of infected leukopenic rabbits were significantly

Table 1: Slit lamp examination scores as a measure of corneal inflammation in *P. aeruginosa*-infected leukopenic rabbits

Group[1]	Leukopenic[2]	SLE[3] 16 hr	22 hr	27 hr
1	Yes	3.2 ± 0.6	4.7 ± 0.8	4.8 ± 1.0
2	No	6.3 ± 0.7	9.7 ± 0.8	14.7 ± 1.8

[1] Each group consisted of 3 rabbits, 6 eyes.
[2] Leukopenia induced by injection of cyclophosphamide (75 mg/kg) followed 24 hr later by dexamethasone (4 mg/kg).
[3] Slit lamp examination scores at 16, 22, and 27 hours after inoculation of *P. aeruginosa*; group 1 scores are significantly lower than group 2 scores ($P<0.0001$) at all three time points.

Table 2: *P. aeruginosa* keratitis in leukopenic rabbits

Group[1]	Leukopenia[2] Present	WBC[3]	Inflammatory cells (Log_{10} PMN)[4]	Viable bacteria (Log_{10} CFU)[5]
1	Yes	2.1 ± 0.3	2.6 ± 0.9	6.8 ± 0.1
2	No	10.0 ± 1.3	5.1 ± 0.2	7.0 ± 0.1

[1] Each group consisted of 3 rabbits, 6 eyes.
[2] Leukopenia induced by injection of cyclophosphamide (75 mg/kg) followed 24 hours later by dexamethasone (4 mg/kg).
[3] WBC = number of white blood cells (x 10^6/ml) in peripheral blood on the day rabbits were sacrificed (3 days after injection of cyclophosphamide); group 1 is significantly different from group 2 ($P<0.0005$).
[4] PMN = Log_{10} polymorphonuclear leukocytes per cornea; group 1 is significantly different from group 2 ($P<0.02$).
[5] CFU = Log_{10} colony forming units per cornea; group 1 is not significantly different from group 2 ($P=0.30$).

less inflamed, as judged by SLE, than eyes of infected nonleukopenic rabbits. These results are compatible with observations made by other investigators studying the relationship between leukopenia and ocular inflammation. Harrison et al. (36) and Trinkaus-Randall et al. (14) induced leukopenia in rabbits with nitrogen mustard and noticed a dampened inflammatory response in the eye when corneas were intrastromally injected with pneumolysin and lipopolysaccharide, respectively. Nanda et al. (37) reported a case of *P. aeruginosa* corneoscleritis in an HIV-infected neutropenic patient presenting with minimal symptoms of infection. Hazlett et al (38) induced leukopenia in outbred Swiss-Webster mice with cyclophosphamide and noticed less severe corneal histopathology in *P. aeruginosa*-infected leukopenic mice; however, the majority of these mice died of septicemia.

In conclusion, the host cellular inflammatory response (predominantly an influx of PMN) appears to contribute significantly to the ocular pathology observed in *P. aeruginosa* keratitis, as evidenced by the significantly lower PMN numbers and SLE scores of leukopenic rabbits.

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TECHNICAL REPORT

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Vice President, Pharmaceutical Sciences

Summary: Moxifloxacin was evaluated for its ability to kill *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the rabbit intrastromal model of keratitis. Topically applied moxifloxacin (0.3%) was shown to be equally active as CILOXAN (0.3% ciprofloxacin) in killing *P. aeruginosa* and *S. aureus* in the cornea. These data are very positive, especially in view of the fact that *in vitro* moxifloxacin is approximately 8 times less active than ciprofloxacin against *P. aeruginosa*.

APPENDIX D

1. INTRODUCTION

An *in vivo* rabbit keratitis model has been developed and utilized by Richard O'Callaghan et al. (LSU Medical School) for several years to evaluate and compare ophthalmic formulations of anti-bacterial agents and their ability to eradicate pathogenic bacteria injected intrastromally from the cornea (Hobden, J. A. et al., 1993).

2. METHODS AND MATERIALS

2.1. Formulations of Moxifloxacin

Three different concentrations of moxifloxacin were prepared and tested. The moxifloxacin vehicle (FID 99916) contained boric acid - 0.155%; sodium chloride – 0.85%; disodium EDTA – 0.05%; benzalkonium chloride – 0.006%; pH adjusted to 7.5. The FID numbers for the three moxifloxacin formulations were FID 99905 - 0.2% moxifloxacin; FID 99906 - 0.3% moxifloxacin; and FID 99907 – 0.5% moxifloxacin.

2.2. Details of Infection Models

The experimental design of the infection models used have been published previously (Hobden, J. A. et al., 1993).

3. RESULTS AND DISCUSSION

Three different concentrations of moxifloxacin were evaluated for their ability to kill *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the rabbit intrastromal model of keratitis. In both infection models, conditions of therapy were chosen such that ciprofloxacin did not completely sterilize the cornea; therefore, moxifloxacin could be evaluated as comparable to ciprofloxacin, more active than ciprofloxacin, or less active than ciprofloxacin.

Table 1

Treatment Results From Infected Rabbit Corneas[2]

Treatment Group	Staphylococcus aureus	Pseudomonas aeruginosa	
	Log CFU at 10 hours	Log CFU at 20 hours	SLE scores at 20 hours
Untreated	6.8 ± 0.13	7.4 ± 0.07	11.1 ± 0.58[c]
Vehicle Control	6.7 ± 0.21	7.4 ± 0.04	11.4 ± 0.68[c]
Moxifloxacin – 0.2%	3.8 ± 0.45[a]	5.5 ± 0.28[b]	11.1 ± 0.67[c]
Moxifloxacin – 0.3%	4.1 ± 0.23[a]	3.8 ± 0.69[b]	11.7 ± 0.53[c]
Moxifloxacin – 0.5%	3.9 ± 0.79[a]	2.1 ± 0.31[b]	11.5 ± 0.39[c]

[a]CFU not significantly different from each other ($P \geq 0.17$), but significantly different from the two control treatments ($P \leq 0.0001$)
[b]CFU significantly different from each other ($P \leq 0.03$), as well as the control groups.
[c]SLE scores were not significantly different from each other.

In the case of *S. aureus* infection, the dosing was a single drop topically 9 hours postinfection and corneas harvested at 10 hours postinfection. Significant killing of the *S. aureus* was observed and was comparable to that for CILOXAN (0.3% ciprofloxacin) tested under these conditions.

In the case of *P. aeruginosa* infection, the dosing was a single topical drop every 30 minutes from 16 to 19 hours postinfection and corneas harvested at 20 hours postinfection. A dose dependent killing was observed: 0.3% moxifloxacin was comparable to CILOXAN (0.3% ciprofloxacin) tested under these conditions.

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The Potency, Ocular Penetration, and Safety of Moxifloxacin (as VIGAMOX® Solution), a Topical Ophthalmic Fourth-Generation Fluoroquinolone

Special Supplement to

Survey of Ophthalmology

The Potency, Ocular Penetration, and Safety of Moxifloxacin (as VIGAMOX® Solution), a Topical Ophthalmic Fourth-Generation Fluoroquinolone

Barry A. Schlech, PhD, Editor

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Special Supplement: The Potency, Ocular Penetration, and Safety of Moxifloxacin (as VIGAMOX® Solution), a Topical Ophthalmic Fourth-Generation Fluoroquinolone

Contents

continued

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INTRODUCTION

Ophthalmic Infections and Their Anti-infective Challenges

Eduardo Alfonso, MD,[1] and Julie Crider, PhD[2]

[1]Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, Florida; and [2]Alcon Research, Ltd, Fort Worth, Texas, USA

Abstract. This introduction provides an overview of the succeeding articles contained within this supplement on the new fourth-generation fluoroquinolone antibiotic product, moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX). Moxifloxacin was developed specifically to address the increasing incidence of resistance to earlier-generation antibiotic molecules. Structural modifications to the moxifloxacin molecule have decreased the likelihood of the development of resistant organisms. This antibiotic has been shown to possess greater activity than previous-generation molecules against gram-positive bacteria while maintaining excellent potency against gram-negative organisms and nontuberculous (atypical) mycobacteria. Moxifloxacin ophthalmic solution 0.5% exhibits enhanced bioavailability due to a unique molecular structure that combines high lipophilicity for enhanced corneal penetration with high aqueous solubility at physiological pH. Numerous studies have shown that moxifloxacin ophthalmic solution 0.5% has high potency against a broad range of microbial species and a favorable profile in terms of safety and tolerability. The results presented in this supplement provide additional evidence for the potential benefits of moxifloxacin ophthalmic solution 0.5% in surgical prophylaxis and treatment of sight-threatening infections, such as bacterial conjunctivitis, endophthalmitis and keratitis. (**Surv Ophthalmol 50:**S1–S6, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. antibiotic • anti-infectives • moxifloxacin • penetration • potency • safety • therapy • VIGAMOX®

Antibiotic Therapy

This supplement provides clinicians with comprehensive information regarding a new antibiotic therapy for the prevention and treatment of bacterial ocular infections. The focus of this supplement is the new fourth-generation fluoroquinolone antibiotic product, moxifloxacin ophthalmic solution 0.5% (Vigamox, Alcon Laboratories, Inc., Fort Worth, TX), which was recently introduced for the treatment of bacterial conjunctivitis. Preclinical study results from *in vitro* and animal experiments as well as results from human clinical trials and postmarket studies will be discussed. These results will provide evidence for the broad utility of moxifloxacin ophthalmic solution 0.5% in the prevention and treatment of a wide variety of ocular infections.

The increasing number of reports concerning ocular bacterial resistance to earlier-generation fluoroquinolones has prompted the development of more advanced fourth-generation antibiotics.[2,5,9]

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Moxifloxacin interferes with bacterial deoxyribonucleic acid gyrase (topoisomerase II) and topoisomerase IV, which are enzymes involved in deoxyribonucleic acid replication within the bacteria.[26] Moxifloxacin is more balanced than earlier-generation fluoroquinolones in its inhibition of these two enzymes. Therefore, the likelihood of producing resistant organisms is diminished considerably, because two simultaneous mutations are required to establish bacterial resistance.[12,30,34] In addition, moxifloxacin possesses a unique bicyclic side chain at the C-7 position that inhibits the efflux mechanism of the bacterial cell and results in rapid death of the target microorganism.[26] The advances in the molecular structure of moxifloxacin provide it with greater potency against gram-positive organisms than was seen with earlier-generation fluoroquinolones, while maintaining similar activity against gram-negative bacteria. The issue of potency and efficacy is covered in detail in this supplement in Schlech and Alfonso's article.[28]

The increasing number of ocular surgical procedures is associated with an increasing risk for perioperative infection. Recent reports indicate that the incidence of bacterial infections after cataract and refractive surgery may be rising.[6,15,24] The risk of surgical complications, such as postoperative endophthalmitis and keratitis, underscores the need for potent new-generation antibiotics for the prevention and treatment of these potentially serious ocular infections. Recent guidelines by the Medicare National Surgical Infection Prevention Project indicate the benefits of preoperative and postoperative antibiotic therapy.[4]

Potency and Efficacy of Moxifloxacin Ophthalmic Solution 0.5%

IN VITRO POTENCY

The Endophthalmitis Vitrectomy Study demonstrated that approximately 94% of isolates from postoperative endophthalmitis are gram-positive bacteria.[10] Therefore, a molecule with greater potency against these pathogenic organisms may provide a therapeutic benefit in the prophylaxis of bacterial endophthalmitis. Studies using bacterial isolates have provided useful information regarding the potency of moxifloxacin ophthalmic solution 0.5% against the target bacteria. Mather and colleagues determined the minimum inhibitory concentrations (MICs) for 93 bacterial endophthalmitis isolates for ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin.[21] Overall, moxifloxacin was the most potent fluoroquinolone tested against gram-positive bacteria ($P = 0.05$). Ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin demonstrated similar potencies against most gram-negative bacteria. In studies with bacterial keratitis isolates, Kowalski et al also reported that moxifloxacin had lower MICs for most gram-positive bacteria than ciprofloxacin, ofloxacin, levofloxacin, or gatifloxacin.[19] These investigators also reported that all fluoroquinolone-susceptible *Pseudomonas aeruginosa* were 100% susceptible to the five fluoroquinolones tested. Aliprandis and colleagues showed that moxifloxacin ophthalmic solution 0.5% was equivalent to ciprofloxacin 0.3% for *P. aeruginosa* in an *in vivo* animal infection model.[3]

Mycobacterium chelonae and *Mycobacterium fortuitum* are the two most common species of nontuberculous mycobacteria found in bacterial keratitis cases.[8,13] These atypical pathogenic bacteria are being found with increasing frequency in surgical settings.[25] Moxifloxacin exhibits excellent activity against these organisms, with MIC_{90} values of ≤ 1.6 µg/mL and ≤ 1.0 µg/mL for *M. chelonae* and *M. fortuitum*, respectively.[1] An antibiotic product, such as moxifloxacin ophthalmic solution 0.5%, with a broad-spectrum coverage of gram-positive and gram-negative organisms that also exhibits potency against atypical mycobacteria may be useful for prevention of postoperative ocular infections.

POTENCY AND EFFICACY IN ANIMAL MODELS

Moxifloxacin ophthalmic solution 0.5% has, for the first time to our knowledge, actually demonstrated potency and efficacy in the prevention of postoperative bacterial endophthalmitis.[19,20] Additionally, in experimental *P. aeruginosa* and *Serratia marcescens* rabbit keratitis models, this fluoroquinolone formulation provided significant decreases in colony-forming units.[31] Topical moxifloxacin 0.5% was equivalent in efficacy to vancomycin 50 mg/mL for treating ciprofloxacin-resistant methicillin-resistant *Staphylococcus aureus* rabbit keratitis.[3] In another study by the same investigators, moxifloxacin (0.5%) showed an efficacy similar to that of ciprofloxacin (0.3%) for the treatment of *P. aeruginosa* keratitis in rabbits. Moxifloxacin treatment also produced a 5.8-log reduction in colony-forming units per cornea in an experimental nontuberculous mycobacterial keratitis rabbit model (F1). The results of these studies suggest a use for moxifloxacin ophthalmic solution 0.5% in various ophthalmic surgical settings.

[F1] Caballero AR, Thibodeaux BA, Dajcs JJ, et al: Effectiveness of fluoroquinolone antibiotics for experimental *Mycobacterium chelonae* keratitis [abstract]. Presented at the 2003 Meeting of the Ocular Microbiology and Immunology Group; Anaheim, CA; November 15, 2003.

SUCCESSFUL TREATMENT OF OCULAR INFECTIONS IN HUMAN CLINICAL STUDIES

Moxifloxacin ophthalmic solution 0.5% was evaluated in clinical safety and efficacy trials using twice a day (b.i.d.) or three times a day (t.i.d.) dosing regimens across studies in newborns (neonates), infants and toddlers, children, adolescents, and adults. The clinical trials involved nearly 2,000 patients from 2 days to 93 years of age. These studies showed that moxifloxacin ophthalmic solution 0.5% is a successful clinical therapy, curing the cardinal clinical signs of bacterial conjunctivitis (i.e., bulbar conjunctival injection and conjunctival discharge/exudate) at a rate of up to 94%. The five primary ocular pretherapy pathogens isolated in the studies were *Staphylococcus epidermidis, S. aureus, Streptococcus pneumoniae, Haemophilus influenzae,* and *Chlamydia trachomatis.* In all of the studies, moxifloxacin ophthalmic solution 0.5% was safe for and well tolerated by patients of all ages. Moxifloxacin ophthalmic solution 0.5% is an effective therapy for bacterial conjunctivitis that successfully treats the key signs of the disease while effectively eradicating its associated pathogens. **(F3)**

Ocular Penetration of Moxifloxacin Ophthalmic Solution 0.5%

For successful control of infections in the eye, a topical antibiotic must effectively penetrate the relevant ocular tissues. The avascular nature of the cornea and vitreous, in particular, limits the uptake of antibiotics into these tissues.[32] Moxifloxacin ophthalmic solution 0.5% achieves better penetration into the cornea and other ocular tissues than other fluoroquinolones.[19,29] This enhanced bioavailability is due to the unique molecular structure of moxifloxacin, which combines high lipophilicity for enhanced corneal penetration with high aqueous solubility at physiological pH. The latter property creates a high concentration gradient at the tear film/corneal epithelial interface, providing a driving force for ocular penetration. *In vitro* studies demonstrated higher permeability in Madin-Darby canine kidney cells for moxifloxacin than for ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, lomefloxacin, or gatifloxacin **(F2)**.[27] This permeability was highly correlated with lipophilicity ($R^2 = 0.92$) and corneal permeability ($R^2 = 0.93$), indicating that the Madin-Darby canine kidney cell model is an excellent predictor of corneal penetration.

Numerous *in vivo* studies have been conducted in rabbits to evaluate the penetration characteristics of moxifloxacin ophthalmic solution 0.5%. In a study using excised rabbit corneas, a 3.6-fold higher corneal permeability coefficient was observed for moxifloxacin than for gatifloxacin **(F2)**.[27] In addition, moxifloxacin transversed the cornea twice as fast as gatifloxacin and demonstrated no effect on epithelial tight cell junctions. Another study in rabbits showed that moxifloxacin ophthalmic solution 0.5% was readily absorbed into anterior ocular tissues with concentrations of 12.5, 1.8, and 6.3 µg/g in the cornea, aqueous humor, and iris-ciliary body at 30 minutes, respectively **(F4)**.[27] Additional reports showed corneal concentrations of moxifloxacin that were at least 700-fold above the MIC for fluoroquinolone-susceptible *S. aureus* and *S. epidermidis* and at least 19-fold higher than the corresponding MIC values for fluoroquinolone-resistant strains of these organisms.[22] In a 4-day multiple-dosing protocol, moxifloxacin ophthalmic solution 0.5% produced peak concentrations in aqueous humor, cornea, and vitreous humor higher than those for either ofloxacin or gatifloxacin **(F5)**.[27]

The superior penetration of moxifloxacin ophthalmic solution 0.5% into ocular tissues has been confirmed in studies in humans.[27] In one study, patients who were dosed with moxifloxacin ophthalmic solution 0.5% before undergoing cataract surgery achieved maximal aqueous humor concentrations that were 25- to 30-fold above the median MIC for susceptible *S. aureus* and *S. epidermidis* isolates from clinical cases of endophthalmitis.[16] Patients who were scheduled to undergo vitrectomy surgery were dosed with moxifloxacin ophthalmic solution 0.5% prophylactically to evaluate penetration of the antibiotic into the aqueous humor.[11] Concentrations that far exceeded the MIC_{90} were achieved in the aqueous humor for a broad spectrum of pathogens, including *S. epidermidis, S. aureus, S. pneumoniae, Streptococcus pyogenes, Propionibacterium acnes, H. influenzae, Escherichia coli, Bacillus cereus, Neisseria gonorrhoeae, Proteus mirabilis,* and a number of other organisms. Two other clinical studies showed that aqueous humor antibiotic concentrations were about two- to four-fold higher in cataract patients after topical administration of moxifloxacin 0.5% versus gatifloxacin 0.3%

[F2] Rusinko A, May J, Liao J, et al: A study of the enhanced corneal penetration of moxifloxacin [abstract]. Invest Ophthalmol Vis Sci 45:4907, 2004.

[F3] Alcon Laboratories, Inc; data on file.

[F4] Robertson SM, Sanders M, Jasheway D, et al: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits [abstract]. Invest Ophthalmol Vis Sci 44:1454, 2003.

[F5] Robertson SM, Sanders M, Jasheway D, et al: Absorption and distribution of moxifloxacin, ofloxacin and gatifloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits [abstract]. Invest Ophthalmol Vis Sci 45:4906, 2004.

(F6).[18,29] Moxifloxacin, ciprofloxacin, ofloxacin, levofloxacin, and gatifloxacin concentrations have also been determined in human conjunctiva after a single topical administration.[33] The mean moxifloxacin tissue concentration was approximately six- to seven-fold higher than the corresponding values for ciprofloxacin ($P = 0.0028$). The consistent enhanced penetration of topical moxifloxacin ophthalmic solution 0.5% should provide significant therapeutic benefits over other topical ocular fluoroquinolones.

Safety of Moxifloxacin Ophthalmic Solution 0.5%

Moxifloxacin ophthalmic solution 0.5% was shown to be well preserved and meets as well as exceeds all Food and Drug Administration requirements and United States Pharmacopoeia standards for antimicrobial preservative effectiveness without the need of benzalkonium chloride in the product (F7). *S. aureus, P. aeruginosa, E. coli, Candida albicans,* and *Aspergillus niger* were all reduced to levels below the minimums required. In addition, moxifloxacin ophthalmic solution 0.5% was challenged with additional organisms not required by the United States Pharmacopoeia to measure the preservative effectiveness of the product. Moxifloxacin ophthalmic solution 0.5% was effective against the protozoan *Acanthamoeba,* the atypical bacterium *Nocardia,* and the fungus *Fusarium.* These studies demonstrate that moxifloxacin ophthalmic solution 0.5% as a multidose ophthalmic product can be safely used without the fear of microbial contamination.

Several *in vitro* studies have demonstrated the safety of moxifloxacin ophthalmic solution 0.5%. Yee and colleagues compared the effects of moxifloxacin, levofloxacin, gatifloxacin, and ofloxacin ophthalmic solutions on human corneal epithelial cells *in vitro* (F8).[17] Moxifloxacin exhibited the least amount of cytotoxicity of the antibiotics tested. In studies from the same report of epithelial healing in chickens after PRK, moxifloxacin treatment resulted in significantly smaller wound sizes than levofloxacin 60 and 66 hours after surgery ($P < 0.05$).

In rabbit studies, lower carboxyfluorescein permeability was observed with moxifloxacin ophthalmic solution 0.5% treatment relative to gatifloxacin ophthalmic solution 0.3% (Zymar®, Allergan, Irvine, CA) (F9). Therefore, moxifloxacin ophthalmic solution 0.5% demonstrated superior maintenance of corneal integrity compared with gatifloxacin ophthalmic solution 0.3%. Using confocal microscopy techniques, Jester and colleagues established a correlation between corneal epithelial thinning (from superficial cells loss) and mild ocular irritation.[14] Confocal microscopy studies performed by Kovoor et al in rabbits evaluated the effects of five topical antibiotic products (0.3% ciprofloxacin, 0.3% ofloxacin, 0.5% levofloxacin, 0.3% gatifloxacin, and 0.5% moxifloxacin) on the epithelial surface of the cornea.[18] All products except moxifloxacin ophthalmic solution 0.5% contained 0.005% or 0.006% benzalkonium chloride. Tears Naturale Free (Alcon Laboratories Inc, Fort Worth, TX) was used as a control. The data showed that 0.5% moxifloxacin did not cause a significant change in corneal epithelial cell layer thickness. After 6 days of treatment, all drug-treated groups, except moxifloxacin ophthalmic solution 0.5% and Tears Naturale Free, caused significant thinning of the corneal epithelial layer. In all groups, the corneal stromal thickness was similar to baseline. Conversely, 0.3% ciprofloxacin, 0.3% ofloxacin, 0.5% levofloxacin, and 0.3% gatifloxacin caused significant thinning of the corneal epithelial layer after 7 days of antibiotic treatment.

A subchronic ophthalmic safety study in cynomolgus monkeys used a dosing regimen of 2 drops, six times a day for 16 days, followed by t.i.d. dosing for the remainder of the 3-month study. Treatments were 0% (vehicle), 0.5%, 1.0%, and 3.0% moxifloxacin ophthalmic solutions. No significant findings were observed for any systemic parameters that were measured (F10).[23] Indirect ophthalmoscopic and slit-lamp biomicroscopic examinations were similar for controls and moxifloxacin treated groups. IOP and specular microscopy revealed no findings related to moxifloxacin treatment. In addition, corneal thickness, a sensitive indicator of corneal health, was not affected by administration of moxifloxacin ophthalmic solution, even at the highest concentrations and most extreme regimens.

[F6] McCulley JP, Surratt G, Shine W: 4th generation fluoroquinolone penetration into aqueous humor in humans [abstract]. Invest Ophthalmol Vis Sci 45:4927, 2004.

[F7] Schlech BA, Sutton A, Rosenthal RA, et al: Antimicrobial preservative effectiveness of VIGAMOX [abstract]. Invest Ophthalmol Vis Sci 45:4913, 2004.

[F8] Yee RW, Sorour HM, Yee SB, et al: Comparison of relative toxicity of four ophthalmic antibiotics using the human cornea epithelial cell culture system [abstract]. Invest Ophthalmol Vis Sci 45:4939, 2004.

[F9] Owen GR, Dembinska O, Stout KR, Mendiola MK: Corneal penetration and change in corneal permeability of moxifloxacin versus gatifloxacin [abstract]. Invest Ophthalmol Vis Sci 45:4910, 2004.

[F10] Bergamini MVW, Heaton J, McGee D, et al: A three month topical ocular toxicity study of moxifloxacin ophthalmic solutions in cynomolgus monkeys [abstract]. Invest Ophthalmol Vis Sci 44:4457, 2003.

General-safety pharmacology studies were designed to profile effects of moxifloxacin hydrochloride on all major organ systems. Moxifloxacin hydrochloride produced no overt side effects in the central nervous systems of test animals (rats and mice) at the highest intravenous dose tested (i.e., 30 mg/kg), which is 1,000-fold higher than the maximum daily dose of moxifloxacin ophthalmic solution 0.5%.[25] In conclusion, the safety margin determined in all of the safety pharmacology studies provides strong support that moxifloxacin hydrochloride administered by the topical ocular route is unlikely to promote significant adverse events in human beings.

Human studies have also confirmed the safety and tolerability of moxifloxacin ophthalmic solution 0.5%. Confocal microscopy experiments reported no changes in the number or morphology of corneal epithelium and endothelium for normal patients treated with moxifloxacin ophthalmic solution 0.5% four times daily for 3 days (F11). In cataract surgery patients, Katz et al evaluated the ocular absorption of moxifloxacin ophthalmic solution 0.5% by measuring the concentration of moxifloxacin in the aqueous humor of patients undergoing cataract surgery.[16] The authors also noted that the administration of moxifloxacin ophthalmic solution 0.5% before cataract surgery had no effect on postoperative corneal and conjunctival healing.

Yee and colleagues evaluated the effects of topical moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% ophthalmic solution on corneal wound healing for patients undergoing bilateral PRK.[35] These authors concluded that both products were safe in PRK. Moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3% produced statistically identical results with respect to haze, visual acuity, and rate of corneal wound healing when administered to PRK patients preoperatively and postoperatively.

Durrie and Trattler showed that moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3% were equivalent with respect to all ophthalmologic measures, the quality of vision, and comfort for patients who had undergone laser-assisted *in situ* keratomileusis and laser epithelial keratomileusis surgery.[7] In addition, moxifloxacin ophthalmic solution 0.5% was shown to be as comfortable as a tear substitute in pediatric subjects (F12). In summary, moxifloxacin ophthalmic solution 0.5% exhibits a desirable safety profile that is an important factor in the prevention and treatment of bacterial infections of an ocular surface that may be compromised to various degrees after surgical procedures.

The Future of Ophthalmic Therapy and the Role of Moxifloxacin Ophthalmic Solution 0.5%

Moxifloxacin ophthalmic solution 0.5% represents a major advance in the treatment and prevention of ocular infections. The increased resistance to the earlier-generation fluoroquinolones (primarily from human systemic and animal husbandry use) prompted the development of the new fourth-generation molecules, such as moxifloxacin, in moxifloxacin ophthalmic solution 0.5%. Moxifloxacin possesses a unique molecule structure that provides superior potency, penetration, and safety. Taken together, these strengths make moxifloxacin ophthalmic solution 0.5% a valuable addition to ophthalmologists' armamentarium of antibiotics for the prevention and treatment of ocular infections. This fourth-generation fluoroquinolone is currently approved in the USA, Canada and India for the treatment of bacterial conjunctivitis. The results presented in this supplement provide additional evidence for the potential benefits of moxifloxacin ophthalmic solution 0.5% in surgical prophylaxis and treatment of sight-threatening infections such as bacterial conjunctivitis, keratitis, and endophthalmitis.

Method of Literature Search

We performed a literature search for this article based on MEDLINE database searches from 1990 to 2005, using various combinations of the search terms *ocular infections, ophthalmic infections, ophthalmic antibiotics, fluoroquinolones, Vigamox, Zymar, moxifloxacin, gatifloxacin, therapy,* and *prophylaxis*. Relevant English journal articles and/or abstracts were selected for review.

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Overview of the Potency of Moxifloxacin Ophthalmic Solution 0.5% (VIGAMOX®)

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Abstract. Antibiotics have been the mainstay of therapy for infectious diseases since their origins in the 1940s. As microorganisms changed and resistance developed, more advanced antibiotics were ultimately needed to provide adequate coverage and spectrum. By selecting optimal antibiotics and dosing regimens, clinicians can avoid treatment failures and adverse events and can help prevent the emergence of further antibiotic resistance. The fourth-generation ophthalmic fluoroquinolones include moxifloxacin (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX) and gatifloxacin (Zymar, Allergan, Irvine, CA), and they are now approved for the treatment of bacterial conjunctivitis. This review highlights four scientific methods that compare and rank antibiotic potencies and predict their clinical efficacy and their propensity to develop resistance: 1) *in vitro* assay for minimum inhibitory concentrations, 2) *in vivo* models for pharmacokinetic and pharamacodynamic properties, 3) therapeutic index or inhibitory quotient, and 4) *in vitro* assay for mutant prevention concentration. The fourth-generation ophthalmic fluoroquinolones perform well in these assays. Both antibiotics have better *in vitro* activity against gram-positive bacteria than ciprofloxacin or ofloxacin. Moxifloxacin penetrates better into ocular tissues than gatifloxacin and older fluoroquinolones; *in vitro* activity of moxifloxacin and gatifloxacin against gram-negative bacteria is similar to that of older fluoroquinolones. Moxifloxacin also has better mutant prevention characteristics than other fluoroquinolones. These findings support the use of the newer fluoroquinolones for the prevention and treatment of serious ophthalmic infections (e.g., keratitis, endophthalmitis) caused by susceptible bacteria. (Surv Ophthalmol 50:S7–S15, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. antibiotic • breakpoints • fluoroquinolone *in vitro* • *in vivo* • MIC • moxifloxacin • pharmacodynamics • pharmacokinetic • potency • MPC • therapeutic index • VIGAMOX®

Since the 1940s, antibiotics have been the mainstay of therapy for infectious diseases. Newer antibiotics with different molecular structures were ultimately needed to overcome the resistance developed by the microorganisms to earlier antimicrobial compounds. For ophthalmology, the story is much the same. With the emergence of antibiotic-resistant organisms, it is essential that clinicians prescribe antibiotics and dosing regimens that will effectively prevent and treat specific sight-threatening ocular infections, such as endophthalmitis and keratitis. To do so, they need to know which antibiotics are most effective against common ocular pathogens. By selecting optimal antibiotics and dosing regimens, clinicians can avoid

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treatment failures and adverse events and can help to prevent the emergence of further antibiotic resistance.

The New Fluoroquinolones

The fourth-generation ophthalmic fluoroquinolones include moxifloxacin (VIGAMOX®, Alcon Laboratories, Inc. Fort Worth, TX) and gatifloxacin (Zymar®, Allergan, Irvine, CA), and they are now approved for the treatment of bacterial conjunctivitis. These new fluoroquinolones are being used off-label by ophthalmologists to prevent bacterial endophthalmitis in patients undergoing surgery.[1,20,28,41] These agents are also commonly being used off-label to treat bacterial keratitis.[24] These new agents provide better coverage against gram-positive organisms and atypical mycobacteria than previously available antibiotics; are more potent, especially against resistant gram-positive pathogens; and delay the emergence of antibiotic-resistant pathogens (F1).[6,23]

Predicting Fluoroquinolone Efficacy

We are now seeing the results of a number of clinical trials that directly compare the efficacies of the new fluoroquinolones to those of older generations of drugs for topical ophthalmic use.[35,42] Such trials guide clinicians in evaluating their relative potency and help them determine which product to recommend for patients. However, there are four scientific methods available to clinicians to help them compare and rank antibiotic potencies of fluoroquinolones and predict their clinical efficacy or their propensity for resistance development.

IN VITRO ASSAY OF MINIMUM INHIBITORY CONCENTRATIONS (MICs)

The MIC involves an *in vitro* determination of antibiotic potency against specific pathogens by comparing the inhibitory activity of various concentrations of a drug against a known inoculum of bacteria (e.g., 10^5 colony-forming units/mL).[4] Other *in vitro* assays assess the killing capability of antibiotics against particular organisms. For example, Stroman and colleagues (F2) conducted *in vitro* studies using clinical isolates of *S. pneumoniae* obtained from patients with bacterial conjunctivitis. Moxifloxacin demonstrated faster kinetics of kill for *S. pneumoniae* than either tobramycin (Tobrex®, Alcon Laboratories, Inc., Fort Worth, TX) gentamicin (Genoptic®, Allergan, Inc., Irvine, CA), or trimethoprim/polymyxin (Polytrim®, Allergan). These *in vitro* data suggest that the faster kill provided by moxifloxacin prevents the spread of infection and therefore decreases the contagiousness of bacterial conjunctivitis.

IN VIVO PHARMACOKINETIC AND PHARMACODYNAMIC MODELS

These models evaluate the penetration and distribution of a drug via one mode of administration into ocular tissues. They consider the *in vitro* MIC of an antibiotic with the drug's *in vivo* pharmacokinetic properties to more accurately predict clinical cures.[22] For example, Wagner and colleagues determined the concentrations of several fluoroquinolones in human conjunctival tissue.[35,42] Twenty minutes following instillation of a single dose, the conjunctival tissues concentrations (μg/g) were: moxifloxacin (18.0), gatifloxacin (2.54), ofloxacin (1.26), ciprofloxacin (2.65) and levofloxacin (2.34). Moxifloxacin demonstrated significantly ($P < 0.0001$) greater penetration to the target tissue than did the other fluoroquinolones.

THERAPEUTIC INDEX OR INHIBITORY QUOTIENT

This index combines MIC data with *in vivo* concentration data at the actual site of infection in tissues and fluids and is, therefore, potentially the most accurate predictor of *in vivo* antibiotic efficacy.[27] Several studies have used MICs and pharmacodynamic models to compare and contrast the potency of the fourth-generation fluoroquinolones with that of earlier generations.[10,20]

IN VITRO ASSAY FOR MUTANT PREVENTION CONCENTRATION (MPC)

The MPC is a novel *in vitro* measurement that determines the propensity of an antimicrobial compound to select for antimicrobial-resistant subpopulations when high-density bacterial inocula (e.g., $>10^9$ organisms) are exposed to various drug concentrations. In human infectious diseases, the number of organisms present at the site of infection is large and may exceed the spontaneous mutational frequency rate, and as such, there may be substantial resistant subpopulations present in high-density cultures. This technology has been applied mostly to the fluoroquinolones and gram-positive organisms. Because resistance to fluoroquinolones occurs in a stepwise fashion, with a single mutation being a first-step resistant mutant and a second mutation representing a second-step resistant mutant, the MPC approach defines the drug concentration required to block the growth of organisms containing first-step resistance mutations. As such, MPC testing is relevant

[F1] Pharmaceutical Association. New Product Bulletin: Avelox™ (moxifloxacin HCL). Washington, 2000.

[F2] Stroman et al unpublished data, 2005.

only with organisms that are determined to be susceptible to the antimicrobial agent by traditional susceptibility testing.[7]

The following discussion is intended to assist ophthalmologists in understanding these four methods of evaluating antibiotic potency, interpreting data from studies using these methods, and applying study results in their clinical practices.

MICs and MPCs: Evaluation of *In Vitro* Antimicrobial Potency and Antibiotic Resistance Development

The MIC is an *in vitro* determination of the lowest concentration of a specific antibiotic that inhibits the growth of an organism at a defined inoculum of bacteria (usually 10^5 colony-forming units/mL).[4] MPCs are another measure of potency and reflect on the antibiotic's robustness in preventing mutant or resistant strain development.[7,25]

DETERMINING MICs

The MIC of an antibiotic for a particular strain of bacteria is determined by standardized microbiological agar and broth tests. The MIC_{90} represents the antibiotic concentration that inhibits 90% of the isolates tested and is calculated when there are at least 10 isolates of a particular microorganism. Likewise, the MIC_{50} represents the antibiotic concentration that inhibits 50% of the isolates tested and can be calculated when there are at least five isolates. In MIC tests, surviving microorganisms are detected by their ability to produce visible growth either on a series of agar plates (i.e., agar dilution or agar diffusion methods) or in microtiter plate wells of broth (i.e., microbroth dilution tests).

Agar Dilution Method

In the agar dilution method, Petri dishes are filled with growth media containing various concentrations of antibiotic and solidified with agar. A defined amount of test organism is inoculated onto the top surface of the solid medium. After incubation, at the proper temperature and atmosphere, for approximately 18–24 hours, the plates are screened for growth. The lowest drug concentration preventing growth is the MIC.

Agar Diffusion Methods

Two other agar-based tests use agar that does not have the antibiotic incorporated into the media. For the Kirby-Bauer (disk diffusion) test, an antibiotic impregnated paper disk is placed on top of the agar that has been inoculated with bacteria. Alternatively, a paper strip containing increasing concentrations of antibiotic is placed on top of the seeded agar (an E test). For both Kirby-Bauer and E tests, the agar plates are incubated overnight to allow the bacterial inoculum to grow and form a continuous dense film of growth on top of the agar, except where growth of the bacterial isolate is inhibited by the antibiotic. This visible zone of inhibition around the disk is measured, and the zone size (Kirby-Bauer) is used to estimate the relative susceptibility or resistance of the organism to the antibiotic. The E test yields an actual MIC. The bacterial strain is reported as sensitive, intermediate, or resistant, depending on the zone size or the MIC.

Broth Dilution Methods

Similar antibiotic dilutions and microorganism challenges are performed using microbiological broth in test tubes or microtiter plates containing various concentrations of antibiotic. Bacterial growth or no growth is measured after incubation and MICs are determined.

Semiautomated instruments may also be used to determine an organism's susceptibility to an antimicrobial compound. The antibiotic with the lowest MIC, MIC_{90}, or MIC_{50} for a particular bacteria is more potent than those antibiotics with higher MICs.[24] However, the *in vitro* MIC data need to be considered along with achievable and sustainable drug concentrations at the site of infection (i.e., various pharmacokinetic and pharmacodynamic parameters). The true killing potential of an antibiotic can be determined in time kill studies, because MICs are a measure of inhibition of bacterial growth. Theoretically, however, the more potent an antibiotic, the less likely the antibiotic will be present at sublethal concentrations and result in partial bacterial killing and, thus, possibly induce resistance.[24] To ensure the therapeutic efficacy of an antibiotic *in situ*, it is necessary to maintain its concentration above the MIC. For concentration-dependent antibiotics, like moxifloxacin, this desired concentration is usually at least 8–10 times the MIC. For concentration-independent or time-dependent antibiotics, like vancomycin, it is the time above the MIC that is more important.

ANTIBIOTIC BREAKPOINTS

The primary function of *in vitro* antimicrobial susceptibility testing in clinical diagnostic laboratories is to provide information to clinicians to guide their selection of antibiotics for therapy. Antibiotic susceptibility or MIC testing is used in clinical research to determine the *in vitro* activity of new antibiotics and to track the incidence and prevalence of antimicrobial resistance.[22] The data from MIC testing are the basis for generating standardized breakpoints (i.e.,

cutoff values) that categorize particular organisms as susceptible, intermediate, or resistant to specific antibiotics. Although somewhat arbitrary, the MIC breakpoints reflect the safe, achievable plasma or serum concentrations of antibiotics and have been established by the National Committee for Clinical Laboratory Standards,[26] Food and Drug Administration, European Medicines Evaluation Agency,[18] and other groups, such as the International Society of Anti-infective Pharmacology. An organism is defined as susceptible to an antibiotic if its MIC is below the serum breakpoint. Infections caused by that particular organism may be effectively treated by standard doses of the antibiotic, which typically achieve concentrations many times the MIC value in the serum.[8,9,24] Organisms demonstrate intermediate resistance if the MIC falls between the susceptible and resistant breakpoints. Infections caused by these pathogens may, in some cases, be effectively treated with higher antibiotic doses that achieve higher serum and tissue concentrations. Pathogens are considered resistant to the antibiotic if the MIC is at or above the established breakpoint and if infections may not be appropriately treated by even achievable concentrations of that antibiotic at that site.[16] For an organism isolated from a particular infection to be susceptible to oral formulations of gatifloxacin, levofloxacin, moxifloxacin, or ofloxacin, the drug must have an MIC of 2 μg/mL or less. However, to be classified as susceptible to ciprofloxacin, the organism must demonstrate a ciprofloxacin MIC of 1 μg/mL or less. Each genus of organism (e.g., *Staphylococcus, Streptococcus*) may have different breakpoints. The antibiotic must be present in the affected tissue at concentrations that are usually many times the MIC for a particular organism.[8,9,24] Again, these antibiotic breakpoints were determined based on the concentration of the antibiotic found in the serum of patients on parenteral or oral therapy. Their direct relevance to topically applied antibiotics in ophthalmology is uncertain, but they represent references for ophthalmologists in using antibiotic preparations in their practices.

Some antibiotics do not have assigned breakpoints for every organism. For example, ciprofloxacin does not have an assigned breakpoint for *Streptococcus pneumoniae.*

COMPARING THE POTENCIES OF FLUOROQUINOLONES

Two recent studies[20,24] determined MIC values for second- (ciprofloxacin and ofloxacin), third- (levofloxacin), and fourth-generation (moxifloxacin and gatifloxacin) fluoroquinolones against endophthalmitis and keratitis isolates and used these values to rank their potencies. Mather et al[24] determined the median MICs for 93 bacterial endophthalmitis isolates using the E-test approach. The antibiotic susceptibility of each infectious isolate was determined by comparing the MIC of each antibiotic to National Committee for Clinical Laboratory Standards for each fluoroquinolone. It should be noted that fewer than 10 organisms were tested for some of the genera/species reported in this study. This is why a median MIC calculation (i.e., MIC_{50}) is the most appropriate method for assessing potency in this particular study. As previously stated, susceptibility to ciprofloxacin is indicated by an MIC of 1 μg/mL or less for most organisms, whereas susceptibility to gatifloxacin, levofloxacin, moxifloxacin, or ofloxacin is indicated by an MIC of 2 μg/mL or less. The results of the study demonstrated that strains of *Staphylococcus aureus* resistant to second-generation fluoroquinolones were more susceptible to moxifloxacin than to gatifloxacin and levofloxacin ($P = 0.01$). Based on these MIC determinations, the potencies of ciprofloxacin, gatifloxacin, levofloxacin, and moxifloxacin were ranked against gram-positive and gram-negative bacteria, including strains resistant to the older fluoroquinolones.

Compared with the second- and third-generation agents, both moxifloxacin and gatifloxacin had superior MIC activity against fluoroquinolone-susceptible and fluoroquinolone-resistant gram-positive bacterial strains isolated from endophthalmitis cases. Ciprofloxacin and levofloxacin were equally potent against gram-positive bacteria. With the exception of two isolates, ciprofloxacin and levofloxacin were more potent than ofloxacin. In comparing the median MICs for the two fourth-generation agents, moxifloxacin was more potent than gatifloxacin against fluoroquinolone-resistant and fluoroquinolone-susceptible *S. aureus* as well as fluoroquinolone-susceptible coagulase-negative staphylococci, *Streptococcus* species (including *S. pneumoniae* and *S. viridans*), and *Enterococcus* species. Moxifloxacin and gatifloxacin were equally potent against fluoroquinolone-resistant coagulase-negative *Staphylococcus* and *Bacillus* species.

In studies by Kowalski et al.[20] and Hwang,[15] both median MICs and MIC_{90}s of conjunctivitis and keratitis isolates were determined and the potencies of fluoroquinolones were ranked against sensitive and resistant gram-positive and gram-negative organisms. Moxifloxacin and gatifloxacin demonstrated lower MIC_{90}s than ciprofloxacin, levofloxacin, or ofloxacin against gram-positive bacteria (MIC ranges of 0.03–2 μg/mL for moxifloxacin/gatifloxacin vs. 0.19–32 μg/mL for ciprofloxacin/ofloxacin/levofloxacin).[15,20] Moxifloxacin and gatifloxacin also demonstrated better *in vitro* susceptibility for isolates

resistant to second- and third-generation fluoroquinolones and equal susceptibility for gram-negative organisms when compared with the older-generation agents, with the exception of *Pseudomonas aeruginosa*. When the *in vitro* activities of the newer-generation agents were compared, moxifloxacin demonstrated statistically better activity (i.e., lower MIC) than gatifloxacin against fluoroquinolone-resistant strains of *S. aureus*, and gatifloxacin demonstrated lower MICs against fluoroquinolone-susceptible isolates of some gram-negative bacteria *(P. aeruginosa, Moraxella, Haemophilus)* (F3). A Study by Stroman et al[20] described the bacteria isolated from bacterial conjunctivitis cases in the USA., Europe and India and determined the antibiotic susceptibility patterns of over 2,300 isolates. They found that resistance to the fourth-generation fluoroquinolones was very low in all three regions for *Staphylococcus epidermidis* isolates (F4).

The apparent weakness of these antibiotics against *P. aeruginosa* is controversial. Through traditional *in vitro* susceptibility studies, moxifloxacin has been found to be less effective than ciprofloxacin against *P. aeruginosa*.[34,40] Nevertheless, Kowalski et al showed that, although there are differences in MICs, all fluoroquinolone-susceptible *P. aeruginosa* were 100% susceptible to the five tested.[20] Also, in rabbit keratitis studies with *P. aeruginosa*, Aliprandis et al showed that moxifloxacin ophthalmic solution 0.5% was equal to ciprofloxacin ophthalmic solution 0.3% (the gold standard for anti-*Pseudomonas* activity).[2] In addition, Dalhoff generally recognized that ciprofloxacin and ofloxacin are highly active against aerobic or facultative gram-negative bacilli. These fluoroquinolones have concentration-dependent killing rates for gram-negative organisms. Earlier fluoroquinolones are not as active against gram-positive bacteria as they are against gram-negative bacteria. Newer fluoroquinolones, such as moxifloxacin, have enhanced activity against gram-positive bacteria but maintain their activity against gram-negative bacteria.[10]

Fourth-generation fluoroquinolones have a broader spectrum of activity because their molecular structures differ from those of older fluoroquinolones. The molecular structures of moxifloxacin and gatifloxacin have greater binding affinity for and thus inhibit two of the enzymes necessary for bacterial deoxyribonucleic acid synthesis (deoxyribonucleic acid gyrase [also called topoisomerase II] and topoisomerase IV) in both gram-negative and gram-positive microorganisms.[19,29] The older fluoroquinolones adequately inhibit deoxyribonucleic acid gyrase in gram-negative organisms but are not as effective as fourth-generation agents for inhibiting topoisomerase IV in gram-positive organisms.[6] The specificity of this mechanism has important implications in the development of resistance to older fluoroquinolones and in ranking the efficacy of newer fluoroquinolones against resistant strains. Data from trials in resistant mutant species suggest that the newer, fourth-generation fluoroquinolones, such as moxifloxacin and gatifloxacin, have a dual-binding mechanism of action, inhibiting both deoxyribonucleic acid gyrase and topoisomerase IV, in gram-positive species. Owing to the rarity of double mutations (e.g., 10^{-14} for fluoroquinolones in *S. pneumoniae*), the preferential use of such agents could limit the emergence of fluoroquinolone resistance.[5] Another indicator of potency, the MPC, can be used to measure the propensity of an antibiotic to encourage resistance development and can help rank antibiotic potency.. Using the MPC approach, Blondeau et al found that for clinical isolates of *S. pneumoniae*, the rank order of antibiotic potencies was moxifloxacin > gatifloxacin > levofloxacin, and for methicillin susceptible *S. aureus*, it was moxifloxacin > gatifloxacin = levofloxacin.[7]

In addition, because of its unique chemical structure, moxifloxacin is a poor substrate for the efflux pump in *S. aureus*.[17,31] The addition of an azabicycloamine side chain on the moxifloxacin molecule makes it more difficult to pump moxifloxacin out of the bacterial cell. This means that once moxifloxacin gets into the bacteria to do its damage, the bacteria has a difficult time pumping out the antibiotic. For a bacterium to resist the lethal effects of moxifloxacin, it must develop more than two mutations or changes to prevent disruption of its two key enzymes, recognize moxifloxacin as a substrate for its efflux mechanism and efficiently pump the antibiotic out of the cell.

LIMITATIONS OF MIC BREAKPOINTS FOR OPHTHALMOLOGY

Although MICs are important *in vitro* indicators of antibiotic activity or potency, they do not necessarily predict *in vivo* antibiotic efficacy. The clinical efficacy of an antibiotic depends on the MIC of the drug relative to the concentration it achieves in target tissues.[21] For example, two antibiotics with an MIC of 1 μg/mL may have different antibacterial effects if one has a peak tissue concentration of 2 μg/mL

[F3] Metzler K, Hedlin P, Blondeau J: Determination of minimal inhibitory concentration (MIC) and mutant prevention concentration (MPC) of ocular isolated of *Pseudomonas aeruginosa* (PA) and *Haemophilus influenzae* (HI) to 5 fluoroquinolone (FQ) antimicrobial agents (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4988, 2004.

[F4] Stroman DW, Cupp GA, Schlech BA: Microbiology of bacterial conjunctivitis (1999–2003). Invest Ophthalmol Vis Sci 46:E.Abstract 5066, 2005.

and the other a peak tissue concentration of 20 μg/mL.[30] As such, it is necessary to compare *in vitro* microbiological measurements with various pharmacological parameters to fully understand and rank antimicrobial agents and their true potencies.

Pharmacokinetics and Pharmacodynamics

For an antibiotic to achieve optimal efficacy, it must reach the pathogen in the infected tissue and remain there for sufficient time at a concentration required for bacterial killing. The pharmacokinetic and pharmacodynamic properties of each antibiotic determine the *in vivo* relationship between the drug and the pathogen necessary to achieve optimal antibiotic efficacy. For ophthalmology, these parameters determine how well the antibiotic penetrates ocular tissues like the cornea, the conjunctiva, or the aqueous humor.

Pharmacokinetics involves the absorption, distribution, and elimination of a drug. An antibiotic's pharmacokinetic properties include measurements of the antibiotic concentrations achieved in a patient's serum or, in the case of ophthalmology, in the ocular tissues. Several parameters can help do this: 1) area under the concentration curve, 2) peak concentration achieved in the tissue (C_{max}), 3) time to maximum concentration in the tissue, 4) elimination half-life, and 5) penetration at different sites of infection. The area under the concentration of a drug is defined as a measure of how much antibiotic reaches the target tissue throughout a set period (usually 24 hours) and helps estimate the drug's bioavailability. When combined with the drug's dosing regimen, pharmacokinetic properties determine the effective time course of drug concentration into ocular tissues. Fluoroquinolones vary in their pharmacokinetic properties.[30,37,38]

Pharmacodynamics describes the relationship between the concentration of a drug over time at the site of infection and the pharmacologic and toxicologic effects of the drug.[13,43] For antibiotics, pharmacodynamic activity depends on the mechanism of action against the bacteria (i.e., bacterial inhibition or killing) and can be described as being either time- or concentration-dependent.[30] For time-dependent antibiotics, such as vancomycin, β-lactams, and macrolides, bacterial killing depends on the amount of time the drug concentration in the ocular tissues exceeds the MIC of the agent.[36] Higher concentrations of these antibiotics do not kill pathogens more rapidly. Therefore, the goal of the dosing regimen of time-dependent drugs is to optimize the duration of drug exposure. For concentration-dependent agents, such as aminoglycosides and fluoroquinolones, higher drug concentrations (i.e., above the MIC) may result in more rapid and extensive bacterial eradication, assuming that the pathogen is sensitive to a given antibiotic. The goal of the dosing regimen with these antibiotics is to maximize drug concentration in the ocular tissues.[8,9] Note that the concentration of the antibiotic in an ophthalmic formulation (e.g., levofloxacin 0.5% or 1.5%) does not affect the MIC of that antibiotic for a given strain. Nevertheless, in these circumstances ophthalmic products with higher concentrations of antibiotics offer better assurance that the antibiotic can be present at or above the MIC for a longer time.

In vitro dynamic studies, animal studies, and a few human studies have been conducted to determine what values for peak:MIC and area under the concentration$_{24}$:MIC (or AUIC) are most predictive of clinical cures for fluoroquinolones. These values could be used to compare fluoroquinolones to determine which would be the most effective in treating infections by a specific organism.[30] These models indicate that a peak:MIC ratio of 10 or higher optimize rapid bacterial killing and prevent regrowth of resistant gram-negative bacterial populations. Nevertheless, some investigators have considered lower AUIC values for moxifloxacin, levofloxacin, and gatifloxacin sufficient to prevent the development of antibiotic resistance (i.e., greater than 30[11,36,44] or greater than 87[12,32]). Animal studies have confirmed the findings of these *in vitro* pharmacodynamic models. In these studies, the AUIC showed the best linear correlation with efficacy.[3]

Because fluoroquinolones demonstrate concentration-dependent killing, a dosing frequency of only once or twice daily will attain a high peak:MIC, whereas administering doses at higher concentrations will produce a high AUIC. A fluoroquinolone regimen of doses given at higher concentrations and infrequent intervals is the most efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. An AUIC value of 75 or above for fluoroquinolones, like moxifloxacin, indicates that these agents would be effective in eradicating specific organisms. In contrast, the favorable pharmacokinetic profiles, particularly the high area under the concentration of ciprofloxacin, ofloxacin, and levofloxacin, are not sufficient to overcome their high MICs.[6,7,25,30]

In addition, peak:MIC and AUIC usually measure serum concentrations of drugs, not drug concentrations at the site of infection. Therapeutic efficacy depends on the concentration of antibiotics at the target site, primarily the concentration of free, or unbound, drug. Most fluoroquinolones rapidly penetrate ocular tissues, achieving tissue concentrations that are generally higher than those found in plasma. Using plasma concentrations as a guide frequently

underestimates the potential for clinical efficacy in ophthamology.[21,30]

Therapeutic Index

The therapeutic index, or inhibitory quotient, is another pharmacodynamic model used to evaluate antibiotic potency. It is based on drug concentration at the ultimate site of action, in tissues rather than in serum, and this makes this methodology relevant to ophthalmology. Higher tissue concentration is a significant factor in determining antibiotic efficacy and preventing resistance.[21] The therapeutic index combines *in vitro* MIC data with *in vivo* penetration data to compare the clinical efficacy of antibiotics (**F5**). The therapeutic index is calculated by dividing the concentration of a drug (e.g., average peak level) that is achievable in the target tissue by the drug's MIC for that pathogen. C_{max} is used to calculate the therapeutic index because the bacterial rate of killing is a function of antibiotic concentration.[27] Therefore, the ratio of C_{max} to MIC and the therapeutic index are the same. An adequate therapeutic index is 1 or higher. Higher values for the therapeutic index indicate greater drug potency or efficacy (**F5, F6, F8**).

Ocular Penetration of Fourth-Generation Fluoroquinolones

Moxifloxacin can be expected to achieve higher concentrations in the tear film and greater penetration into anterior ocular tissues than older fluoroquinolones because it is more soluble at the normal physiologic pH of 7.0 at the ocular surface and is both lipophilic and hydrophilic. Moxifloxacin is formulated at a pH of 6.8, and gatifloxacin at a pH of 6.0. In addition, moxifloxacin is available in a higher concentration than gatifloxacin (0.5% vs 0.3%). In combination, moxifloxacin achieves higher tissue penetration than gatifloxacin. Published data (concentrations achieved by moxifloxacin and gatifloxacin in intact rabbit corneas) suggest real differences in the ocular penetration of these fluoroquinolones (**F7, F8**)[35,39]. A solution of moxifloxacin at 0.3% (i.e., 40% lower or 0.2% less than the commercial formulation) achieved high concentrations in rabbit ocular tissues within 30 minutes after a single dose: 12.5 μg/mL in the cornea and 1.8 μg/mL in the aqueous humor. In contrast, gatifloxacin 0.3% achieved concentrations of 4.5 μg/mL in the cornea and 0.27 μg/mL in the aqueous humor at 1 hour after administration of a single dose. Moxifloxacin reached almost a threefold higher concentration in the cornea compared with gatifloxacin in approximately half the time.

A recent open-label human pharmacokinetic study (**F9**) measured moxifloxacin penetration into the aqueous humor in human adults undergoing cataract surgery. The C_{max} for patients who received topical moxifloxacin preoperatively exceeded the MIC values for *S. aureus* and *Staphylococcus epidermidis*, suggesting that moxifloxacin may be an effective prophylactic antibiotic for endophthalmitis. These findings confirmed the rabbit data (**F8**), which demonstrated that levels up to 1.84 μg/mL of moxifloxacin could be achieved in the anterior chamber after topical administration. Using the human penetration data and the MIC data, the therapeutic index for moxifloxacin against *S. aureus* can be calculated. The C_{max} in the aqueous humor is 1.84 μg/mL divided by 0.06 μg/mL, the MIC for *S. aureus*. Therefore, the therapeutic index for moxifloxacin against *S. aureus* is 30.7, which indicates that topically applied moxifloxacin ophthalmic solution 0.5% achieves therapeutic concentrations in the aqueous humor, delivering 30 times the minimum amount needed to inhibit growth of organisms, like *S. aureus*. Metzler et al recently reported an MIC_{90} value of 0.063 μg/mL and an MPC_{90} value of 0.25 μg/mL against clinical isolates of methicillin-susceptible *S. aureus*.[25] Therapeutic indexes for moxifloxacin using these values would be 29.2 and 7.36, respectively. As such, not only does moxifloxacin deliver substantially more drug than is necessary to inhibit bacterial growth, but it also delivers substantially more drug than required to prevent the selection of resistance by the MPC model.

Limitations of the Therapeutic Index

Moxifloxacin and gatifloxacin penetrate the anterior chamber better than the older-generation agents. This in turn suggests that the therapeutic

[F5] McGreal JA: Therapeutic drugs: are you using the best weapon against bacterial keratitis? Rev Optometry Online 1999. Available at: http://www.revoptom.com/index.asp) viewed June 14, 2005.

[F6] Sheppard JD: A new generation to treat infection. Rev Ophthalmol Online 2003. Available at: http://www.revophth.com/index.asp) viewed June 14, 2005.

[F7] Batoosingh AL, Lee E, Welty DF, Tang-Liu D: Gatifloxacin 0.3% vs. ciprofloxacin 0.3%: ocular pharmacokinetic profile following topical application in rabbits (abstract). Invest Ophthalmol Vis Sci 44(Suppl):2117, 2003.

[F8] Robertson SM, Sanders M, Jasheway D, et al: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration in pigmented rabbits (abstract). Invest Ophthalmol Vis Sci 44(Suppl):1454, 2003.

[F9] Katz HR, Masket S, Lane SS, et al: Human aqueous humor penetration pharmacokinetics of moxifloxacin after topical administration of moxifloxacin 0.5% ophthalmic solution. Abstract 10, presented at meeting of Ocular Microbiology & Immunology Group, 2003.

indexes of moxifloxacin and gatifloxacin is higher than those of older agents. More clinical studies are needed to correlate the therapeutic index with clinical outcomes for specific ocular infections. Because the therapeutic index is calculated using the drug concentration at the site of infection, it may be a better description of antibiotic potency and a more reliable method for comparing antibiotics. However, a drug's therapeutic index does not account for variability in tissue levels, any synergy or antagonism among concomitant drugs, or the time it takes for a drug to reach peak concentration (**F5**).

Mutant Prevention Concentrations

Blondeau et al[7] used MPCs to compare antibiotic potencies of five fluoroquinolones against *Streptococcus pneumoniae.* There was a hierarchy of potency based on MPCs and an antibiotic's ability to prevent the growth of first-step mutants. Moxifloxacin had the lowest MPC and therefore the best activity in this investigation.

Summary

Studies of endophthalmitis and keratitis isolates have shown that fourth-generation fluoroquinolones have MICs against gram-positive organisms lower than those against second- and third-generation agents. Therefore, they have greater *in vitro* potency against these ocular pathogens. Potency against gram-positive organisms is especially important because the majority of ocular infections, particularly postoperative endophthalmitis and keratitis, are caused by gram-positive organisms.[14,33]

Because pharmacodynamic models, primarily peak:MIC and AUIC, consider the *in situ* concentrations of antibiotics, they could provide a more accurate prediction of the clinical efficacy of fluoroquinolones than MIC values alone.[41] However, clinical studies have not been conducted using these models to specifically evaluate the potency of fourth-generation fluoroquinolones against gram-positive ocular infections and to compare those values with clinical outcomes. Because the therapeutic index is calculated using the drug concentration at the site of infection as well as the MIC, it is the most reliable evaluation of antibiotic potency and the best predictor of clinical efficacy among fluoroquinolones used to treat ocular infections. Studies in animals and humans correlated the therapeutic index and other measures of fluoroquinolone potency with clinical outcomes in specific ocular infections. These studies have demonstrated excellent achievable concentrations of moxifloxacin and gatifloxacin in the cornea and aqueous humor (**F7, F8**).[35] These findings, taken in conjunction with the lower MICs found for both agents, indicate that the newer fluoroquinolones may ultimately improve clinical outcomes for patients with serious ophthalmic infections, including endophthalmitis and keratitis (**F10**).

Method of Literature Search

We performed an international literature search for this article based on MEDLINE database searches from 1990 to 2005, using varying combinations of the search terms *ocular infections, ophthalmic antibiotics, fluoroquinolones, moxifloxacin, potency, therapy, prophylaxis,* and *future.* All relevant journal articles and/or abstracts were selected for review. English abstracts were used for non-English papers.

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ELSEVIER

In Vitro and *In Vivo* Potency of Moxifloxacin and Moxifloxacin Ophthalmic Solution 0.5%, A New Topical Fluoroquinolone

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Abstract. Fluoroquinolones are a class of synthetic antibacterial agents that were approved for ocular therapy in 1991 and have become popular therapy for the treatment and prevention of various ocular infections. These agents are synthetic, broad-spectrum, rapidly bactericidal, and have good penetration into ocular tissues. Their main mechanism of action is the inhibition of bacterial enzymes needed for bacterial DNA synthesis. However, antibiotic resistance occurred swiftly to the earlier fluoroquinolones and better fluoroquinolones were needed. The fourth-generation fluoroquinolones, such as moxifloxacin and gatifloxacin, have enhanced activity against gram-positive bacteria while retaining potent activity against most gram-negative bacteria. These fourth-generation fluoroquinolones have improved penetration into the anterior chamber and have also demonstrated increased *in vivo* efficacy in several animal models of ocular infections. In addition, topical ophthalmic antibiotic products can deliver antibiotic concentrations directly to the eye that are thousands of times higher than their MICs. This article reviews published data describing the *in vitro* potency of moxifloxacin and its *in vivo* activity for treating and preventing experimental ocular infections. (**Surv Ophthalmol 50:**S16–S31, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. antibiotic activity • antibiotic potency • antibiotic resistance • *in vitro* • *in vivo* • MICs • moxifloxacin • VIGAMOX®

Introduction

Fluoroquinolones are synthetic, broad-spectrum, bactericidal antibiotics that were approved for treatment of ocular infections in 1991. The effectiveness of second- and third-generation fluoroquinolones (e.g., ofloxacin, ciprofloxacin, levofloxacin) has been offset by the emergence of fluoroquinolone-resistant organisms.[46,68,73] The fourth-generation fluoroquinolones (moxifloxacin and gatifloxacin) show an enhanced spectrum of activity against gram-positive bacteria and comparable activity to second- and third-generation fluoroquinolones (ciprofloxacin and levofloxacin) against gram-negative bacteria.[7,11,12,25,26] New ocular antibiotic formulations with improved potency, such as moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon Laboratories, Fort Worth, TX) or gatifloxacin ophthalmic solution 0.3% (Zymar®, Allergan, Irvine, CA), are currently available and have been shown to inhibit growth of organisms resistant to second- and third-generation

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fluoroquinolones.[78] The purpose of this article is to review a) the *in vitro* activity of moxifloxacin against clinical ocular isolates and b) the *in vivo* effectiveness of moxifloxacin ophthalmic solution 0.5% in treating or preventing experimental ocular infections.

Mechanism of Action

The fluoroquinolones are potent antibacterial agents that target bacterial enzymes necessary for DNA synthesis (i.e., replication, transcription, repair, and recombination). These important bacterial enzymes are DNA gyrase and topoisomerase IV.[10,31–35] The principal event in the action of the fluoroquinolone is the trapping of gyrase or topoisomerase IV on DNA as ternary drug-enzyme-DNA complexes.[31,34,116] The fluoroquinolone-enzyme-DNA complexes prevent uncoiling and/or separation of the replicated strands of DNA, resulting in the inhibition of DNA replication and death of the bacterium.[40,52] The breaks in the double-stranded DNA result in the death of the replicating cell.[32,83,116]

Individual fluoroquinolones target either DNA gyrase or topoisomerase IV and in some cases, both. The DNA gyrase is the target in organisms, such as *Mycobacterium tuberculosis*, *Helicobacter pylori*, and *Treponema pallidum*, which represent a group of bacteria with genomes that lack a gene encoding topoisomerase IV.[33] In gram-negative organisms DNA gyrase is more sensitive than topoisomerase IV to fluoroquinolones and is considered to be the primary target.[9,33,36,37,40,85,126] The target of fluoroquinolones for gram-positive organisms is more complex and varies based on the individual fluoroquinolone, as demonstrated in Table 1 for *Staphylococcus aureus* and *Streptococcus pneumoniae*. The gyrase in these organisms is less sensitive to fluoroquinolones than in gram-negative bacteria.[32] The general target in gram-positive organisms is topoisomerase IV.[9,36,37,40,85,109,122] This enzyme tends to have about the same sensitivity in both organisms. The fourth-generation fluoroquinolones target both DNA gyrase and topoisomerase IV.[10,33,38,42,101]

TABLE 1

Inhibitory Activities of Fluoroquinolones Against DNA Gyrase and Topoisomerase IV

Fluoroquinolone	IC_{50} (μg/mL) DNA Gyrase	Topoisomerase IV	IC_{50} Ratio[b]
From *Staphylococcus aureus*[a]			
Ciprofloxacin	13.5	5.76	0.43
Ofloxacin	18.8	22.8	1.21
Levofloxacin	8.06	9.81	1.22
Moxifloxacin	3.44	7.84	2.28
From *Streptococcus pneumoniae*[c]			
Moxifloxacin	8.02 (20 μM)	4.01 (10 μM)	0.5
Gatifloxacin	7.5–15.0 (20–40 μM)	3.6–7.5 (10–20 μM)	0.5
Levofloxacin	28.9 (80 μM)	14.4 (40 μM)	0.5
Ciprofloxacin	13.25 (40 μM)	7.6 (20 μM)	0.5

[a] From Takei M et al.[109]

[b] IC_{50} Ratio: IC_{50} against topoisomerase IV/IC_{50} against DNA gyrase.

[c] Calculated from Yague G et al.[122]

Mechanisms of Resistance

Resistance to fluoroquinolones emerged shortly after the introduction of the second-generation compounds, ofloxacin and ciprofloxacin.[3,46,73] Resistance to fluoroquinolones requires significant genetic changes in one or more of four major bacterial mechanisms: a) enzymes for DNA synthesis, b) gyrase protecting proteins, c) cell permeability, or d) drug efflux.[56,99] Also, enzymes that degrade fluoroquinolones have not been reported in bacteria, but have been found in fungi.[119] Fluoroquinonolone resistance develops in a step-wise fashion. Lowered susceptibility has been associated with porins that regulate intracellular drug concentration or changes in proteins that protect target enzymes from attack. These changes may occur spontaneously and the mutants are subsequently selected by suboptimal fluoroquinolone therapy.[93] Much of the resistance has been caused by the systemic, agricultural, and vetinary use of fluoroquinolones and less as a result of topical administration. Wegener and Engberg provided an excellent review of the past veterinary use of quinolones, and discourage such use to preserve the efficacy of quinolones for human use.[118] Ciprofloxacin has been licensed for use in swine and chickens in Asia and Latin America. Ofloxacin has been licensed for use in chickens and turkeys in Japan and Asia. Topical application of fluoroquinolones tends to produce less resistance because the concentration of drug introduced to the site of infection is several hundred, and often several thousand times, the MIC against common organisms. The ability of topical antibiotic products for the eye, such as moxifloxacin ophthalmic solution 0.5%, to deliver 5,000 μg/ml of antibiotic directly onto the infected tissue is formidable. The 5,000 μg/ml is 10,000 times the MIC of 0.5 μg/ml, a common MIC for this antibiotic against ocular isolates.

The following discussion reviews each of four major mechanisms by which organisms become resistant to a particular antibiotic.

CHANGES IN ENZYME TARGETS

Point mutations in the genes encoding DNA gyrase or topoisomerase IV reduce the affinity of the fluoroquinolones to these enzymes.[90] DNA gyrase is a complex of GyrA and GyrB subunits encoded by the *gyrA*

and *gyrB* genes that introduce supercoiling into DNA in a reaction driven by the hydrolysis of ATP. DNA gyrase is essential for initiation of DNA replication and has a role in elongation by removing positive supercoils from DNA as a result of unwinding at the replication fork. Topoisomerase IV is also involved in DNA replication by the decatenation of linked daughter chromosome during the end stages of replication. Topoisomerase IV is composed of two subunits ParC and ParE that are encoded by the *parC* and *parE* genes, respectively.

Resistance in gram-negative bacteria occurs typically as a result of alterations in DNA gyrase, either in the GyrA or GyrB subunit.[126] Mutations in the GyrA subunit have a tendency to cluster in the quinolone resistance-determining region (QRDR). QRDR represents the region of the *gyrA* gene, which encodes the GyrA subunit that binds to DNA during enzyme activation.[126] Mutations in QRDR are thought to cause resistance through decreased drug affinity for the altered gyrase-DNA complex.[120] Mutations to the GyrB subunit occur less frequently than mutations to the GyrA subunit. Whether GyrB mutations affect fluoroquinolone binding remains unclear;[125] however, alterations in GyrB subunit produce lower levels of resistance as compared to GyrA subunit mutations.[55]

Fluoroquinolone resistance in gram-positive organisms generally result from mutations to topoisomerase IV subunits by alterations to the ParC or ParE subunits, with mutations in ParC playing a more prominent role in resistance.[39,85] Many fluoroquinolones primarily target topoisomerase IV of *S. aureus*, but the target in *S. pneumoniae* varies among the fluoroquinolones.[87,88,109,122] Furthermore, the fourth-generation fluoroquinolones were reported to target both DNA gyrase and topoisomerase IV.[10,33,38,42,87,89,101] Topoisomerase IV mutations have been studied in gram-negative bacteria and are believed to play a secondary role in development of resistance as ParC or ParE mutations typically confer resistance only in the presence of concomitant DNA gyrase mutations.[17,63]

Genetic studies have shown that DNA gyrase is the primary target of quinolones in *Escherichia coli*. In genetic studies, single mutations in either GyrA or GyrB subunits of DNA gyrase conferred first-step and subsequent incremental drug resistance.[51] Additional studies demonstrated that topoisomerase IV is a secondary drug target in *E. coli*.[63] Genetic studies with *S. aureus* showed that first-step drug resistance was found in the mutants with point mutations to the ParC or ParE subunits of topoisomerase IV, indicating topoisomerase IV is the primary target of most quinolones.[39,85,109] The genetic data are not as straightforward for other species, however, there does appear to be a pattern that shows DNA gyrase is the primary quinolone target in gram-negative bacteria and that topoisomerase IV is the primary drug target in gram-positive bacteria.[56]

PRODUCTION OF GYRASE PROTECTION PROTEIN

All of the genetic loci and mutations that confer resistance to fluoroquinolones are chromosomally mediated with the exception of a plasmid-mediated gyrase-protecting protein recently described in *Klebsiella pneumoniae*.[76,95] The plasmid found primarily in gram-negative organisms contains the *qnr* (quinolone resistance) locus that confers resistance by encoding a 218-amino acid protein that protects DNA gyrase from the fluoroquinolones.[113] The frequency of clinical isolates containing the *qnr* determinant is unknown.[95]

DECREASING CELL PERMEABILITY

In order for fluoroquinolones to access their targets in the cytoplasm, they must traverse the cell wall and cytoplasmic membrane of all bacteria and the outer membrane of gram-negative organisms. The cell wall of gram-positive organisms is believed not to be a barrier to diffusion of fluoroquinolones and other small molecules of 300–400 Da. Porins in the outer membrane are responsible for regulating drug diffusion. However, in gram-negative organisms, decreased levels of porins in the outer membrance reduce the accumulation of fluoroquinolone in the cytoplasm.[53,92]

EFFECTIVE EFFLUX PUMPS

The bacteria's efflux pump mechanism contributes to bacterial resistance by preventing lethal levels of fluoroquinolone from accumulating in the cytoplasm.[55] The efflux pump is a mechanism that expels the fluoroquinolone across the cell membrane and out of the cell, thereby reducing the intracellular concentration to sublethal levels.[27,55] The action of the efflux pump is dependent on the ability of fluoroquinolones to bind to the bacterial efflux protein which expells it from the cell. Some fluoroquinolones, particularly moxifloxacin, are less affected by bacterial efflux mechanisms due to their bulky side-chain moiety at position 7 that hinders its export out of the cell.[91]

CONCLUSION

Mutations that confer resistance to second- and third-generation quinolones also lower susceptibility to fourth-generations quinolones. An isolate with reduced susceptibility to one fluoroquinolone will be less susceptible to all fluoroquinolones; however,

the reduced susceptibility may or may not be above the breakpoint definition of resistance. Gram-positive bacteria are less likely to be resistant to the fourth-generation fluoroquinolones due to their enhanced potency. The second- and third-generation fluoroquinolones can select for mutations to either DNA gyrase or topoisomerase IV and produce a situation where bacteria will need only one additional mutation to become resistant to the fourth-generation fluoroquinolones. The preferential use of the fourth-generation fluoroquinolones for gram-positive ocular infections may delay the emergence of resistant isolates. Bacteria are less likely to develop resistance to the fourth-generation fluoroquinolones than the second-generation due to the dual targeting.

In Vitro Potency

IN VITRO SUSCEPTIBILITY OF BACTERIA RECOVERED FROM OCULAR INFECTIONS

Moxifloxacin has been shown to possess potent *in vitro* activity against a wide spectrum of bacteria and is more active against *Staphylococcus* and *Streptococcus* species than previous generation fluoroquinolones.[7,41,45,54] Table 2 (gram-positives), 3 (gram-negatives), and 4 (atypicals) present a comparison of intrinsic susceptibilities to fluoroquinolones for bacterial species routinely encountered in ocular infections. Intrinsic susceptibility to an antibiotic is typified by the median (50%) minimal inhibitory concentration of the organisms tested (MIC_{50}), unless more than 50% of the isolates of the specific species have acquired resistance to the antibiotic. Intrinsic susceptibility to a specific antibiotic for a particular species is the same regardless from where the isolates are recovered (i.e., geographical areas or sites of infection). This allows comparisons of fluoroquinolone susceptibilities of bacteria from different sites of infection.

Several studies have compared the activity of moxifloxacin and other fluoroquinolones against clinical bacterial isolates from ocular and nonocular sites.[7,12,41,45,54,67,108] Table 2, 3, and 4 show comparative *in vitro* data, which generally demonstrate that moxifloxacin is more active than the earlier generation fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin), especially against staphylococci, streptococci, and a variety of atypical organisms.[100] Based on their reported MICs against atypical mycobacteria, like *Mycobacterium chelonae, M. kansasii,* and *M. fortuitum,* moxifloxacin and gatifloxacin exhibit more favorable *in vitro* susceptibility results than do ciprofloxacin, levofloxacin, and ofloxacin (Table 4).[1,2,44,96,100] Moxifloxacin has been shown to be slightly more active than gatifloxacin against staphylococci and streptococci.[7,41]

FREQUENCY AND SIGNIFICANCE OF RESISTANT ISOLATES IN OCULAR INFECTIONS

After the introduction of ciprofloxacin into medical practice in 1985–1987, reports of ciprofloxacin resistance in *Staphylococcus* appeared almost immediately (within a year).[14,98] The emergence of ciprofloxacin resistance, especially in MRSA strains, in hospitals caused concern for the future usefulness of fluoroquinolones for treating *S. aureus* infections.[28,58,66,74,82,103] Apparently, the tremendous amounts of fluoroquinolones used around the world and its inappropriate use in the past two decades has diminished the initial effectiveness due to the rapid emergence of fluoroquinolone-resistant strains. By the time ciprofloxacin ophthalmic solution 0.3% (Ciloxan®, Alcon Laboratories, Fort Worth, TX) was introduced to the American market in 1991, the fluoroquinolone resistance rate in *S. aureus* isolates from systemic infections was already 11%[64] and 14% in ocular infections.[3] Furthermore, retrospective studies of clinical keratitis isolates found the resistance rate of *S. aureus* had increased to 28–35% by 1998.[3,46] Ciprofloxacin resistance was reported for gram-positive organisms; however, resistance is less common in gram-negative organisms.[43,64,110]

There are two primary approaches to define antibiotic resistance. The first approach is the breakpoint MIC method. The National Committee for Clinical Laboratory Standards (NCCLS) has defined MIC breakpoints for many antibiotics used to treat infections, especially blood borne, urinary tract, and respiratory tract infections (**F1, F2**). Breakpoint susceptibility testing uses designated antibiotic concentrations necessary to differentiate between the interpretive categories of "susceptible," "intermediate," or "resistant," rather than a range of five or more doubling-dilution concentrations used to determine MICs.[60] A specific breakpoint MIC is defined for each antibiotic, above which if the isolate survives and grows it is considered to be resistant to that antibiotic. The MIC breakpoint usually is predictive of clinical efficacy for systemic therapy because it is set with the antibiotic's specific pharmacokinetic parameters in view. Resistant isolates would likely fail systemic therapy. The second approach is the acquired resistance approach. This approach recognizes the intrinsic susceptibility of a particular species

[F1] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; M100-S15, Fifteenth Informational Supplement. Wayne PA: Jan 2005.

[F2] National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement. Wayne, PA: NCCLS, 2002. Document M100-S12, 2002.

TABLE 2

Susceptibility of Gram-Positive Species to Five Fluoroquinolones

Bacterial Species	MIC_{50} (μg/ml)				
	Moxifloxacin	Gatifloxacin	Levofloxacin	Ciprofloxacin	Ofloxacin
Staphylococci					
Staphylococcus aureus	0.03[a,b,c]	0.06[a], 0.094[b]	0.18[a], 0.19[b]	0.25[a,c], 0.38[b]	0.50[b,c]
Staphylococcus epidermidis	0.06[a,b,c,d]	0.13[a,b,d]	0.19[b,d], 0.50[a]	0.22[b,d], 0.25[c], 0.50[a]	0.50[b,c,d]
Staphylococcus haemolyticus	0.06[a,c]	0.13[a]	0.50[a]	0.50[a,c]	0.50[c]
Staphylococcus saprophyticus	0.03[a], 0.13[c]	0.06[a]	0.25[a]	0.25[a], 0.50[c]	1.0[c]
Staphylococcus lugdunensis	0.13[c]	-	-	0.25[c]	0.50[c]
Staphylococcus hominis	0.03[a], 0.06[c]	0.13[a]	0.50[a]	0.13[c], 0.50[a]	0.25[c]
Staphylococcus simulans	0.03[a]	0.06[a]	0.25[a]	0.25[a]	-
Staphylococcus pasteuri	0.06[c]	-	-	0.25[c]	0.50[c]
Staphylococcus warneri	0.03[a], 0.06[c]	0.13[a]	0.25[a]	0.25[a,c]	0.50[c]
Streptococci and Enterococci					
Streptococcus pneumoniae	0.06[c], 0.13[a,b]	0.22[b], 0.25[a]	0.75[b], 1.0[a]	0.50[c], 0.75[b], 1.0[a]	1.0[c], 2.0[b]
Streptococcus mitis	0.13[c]	-	-	2.0[c]	2.0[c]
Streptococcus, viridans group	0.13[b,c]	0.25[b]	0.75[b]	1.0[b], 2.0[c]	2.0[b,c]
Streptococcus pyogenes	0.13[c], 0.25[a]	0.25[a]	1.0[a]	0.50[c], 1.0[a]	1.0[c]
Enterococcus faecalis	0.19[b,d], 0.25[a,c]	0.38[b,d], 0.50[a]	0.75[b,d], 1.0[a]	0.75[b,d], 1.0[a,c]	2.0[b,d,c]
Micrococci					
Micrococcus luteus	0.50[c]	-	-	1.0[c]	2.0[c]
Kocuria spp.	0.25[c]	-	-	1.0[c]	2.0[c]
Bacilli					
Bacillus cereus	0.09[b,e], 0.13[c]	0.09[b,e]	0.13[b,e]	0.13[b,e,c]	0.25[c], 0.38[b,e]
Bacillus pumilus	0.13[c]	-	-	0.13[c]	0.13[c]
Bacillus subtilis	0.016[c]	-	-	0.13[c]	0.13[c]
Coryneforms					
Corynebacterium accolens	0.03[c]	-	-	0.06[c]	0.25[c]
Corynebacterium macginleyi	0.06[c]	-	-	0.03[c]	0.13[c]
Corynebacterium propinquium	0.25[c]	-	-	0.25[c]	1.0[c]
Corynebacterium pseudodiphtheriticum	0.25[c]	-	-	0.25[c]	1.0[c]

[a] Published data from Bauernfeind et al.[7]
[b] Published data from Kowalski et al[67] and Mather et al.[78]
[c] Unpublished data from Alcon.
[d] Coagulase-negative *Staphylococcus* rather than *Staphylococcus epidermidis*.
[e] *Enterococcus* spp. rather than *Enterococcus faecalis*.
[f] *Bacillus* spp. rather than *Bacillus cereus*.

to an antibiotic and considers any isolate with a 4- to 16-fold increase in MIC above the intrinsic susceptibility to have acquired resistance. There is no implied relationship between isolates defined as resistant by this method and clinical outcome. This method is appropriate when attempting to define genetic changes that confer resistant phenotypes.

Two terms, "MIC_{90}" and "% resistant" are useful in expressing the prevalence of resistant isolates within a group of isolates of a particular species to a specific antibiotic. If less than 10% of the isolates are resistant, the MIC_{90} will be similar to the MIC_{50}. If 10–49% of the isolates within the group are resistant, the MIC_{90} will be higher than the MIC_{50}. If more than 50% of the isolates are resistant, the MIC_{50} will be higher as well. Therefore, the number or frequency of isolates of a particular species that are resistant cannot be derived from the MIC_{90}, but rather must be calculated directly as a percentage of the total number of isolates of the specific species tested.

The frequency of encountering fluoroquinolone-resistant isolates prior to therapy has increased during the last 15 years in several important species recovered from healthy ocular surfaces as well as from ocular infection.[3,15,19,46,57,59,71,75,114,117] Most of these reports define resistance as any isolate with a ciprofloxacin MIC of 2.0 μg/ml or greater. The resistance breakpoint for ofloxacin is 1.0 μg/ml. The current NCCLS resistant breakpoints (**F1**) for moxifloxacin are established for *Staphylococcus* species as greater than or equal to 2.0 μg/ml and for *Streptococcus pneumoniae* as greater than or equal to 4.0 μg/ml. There are no resistant breakpoints for moxifloxacin for any other bacterial species. There is still debate about their relevance.[106]

Significant geographical differences in the frequencies of resistant isolates have been reported in ocular infections.[3,19,46,67,75,97] Particularly important is the rapid emergence of fluoroquinolone- and methicillin-resistant *Staphylococcus aureus* (MRSA) in

TABLE 3

Susceptibility of Gram-Negative Species to Five Fluoroquinolones

Bacterial Species	MIC_{50} (μg/ml)				
	Moxifloxacin	Gatifloxacin	Levofloxacin	Ciprofloxacin	Ofloxacin
Enterobactericeae					
Aeromonas caviae	0.13[c]	-	-	0.03[c]	0.06[c]
Citrobacter koseri	0.03[c]	-	-	0.008[c]	0.06[c]
Enterobacter aerogenes	0.06[a], 0.25[c]	0.06[a]	0.06[a]	0.03[a], 0.13[c]	0.25[c]
Enterobacter cloacae	0.03[a], 0.13[c]	0.016[a]	0.03[a]	0.016[a], 0.13[c]	0.25[c]
Enterobacter hormaechei	0.13[c]	-	-	0.03[c]	0.13[c]
Escherichia coli	0.008[a], 0.06[c]	0.008[a]	0.016[a]	0.008[a], 0.03[c]	0.13[c]
Klebsiella oxytoca	0.03[a], 0.25[c]	0.016[a]	0.03[a]	0.016[a], 0.03[c]	0.13[c]
Klebsiella pneumoniae	0.03[a], 0.13[c]	0.03[a]	0.03[a]	0.016[a], 0.06[c]	0.25[c]
Morganella morganii	0.06[a], 0.50[c]	0.06[a]	0.03[a]	0.016[a], 0.06[c]	0.50[c]
Pantoea agglomerans	0.03[a], 0.06[c]	0.016[a]	0.03[a]	0.016[a], 0.03[c]	0.13[c]
Proteus mirabilis	0.06[a], 0.50[c]	0.13[a]	0.03[a]	0.016[a], 0.03[c]	0.25[c]
Serratia marcescens	0.25[a,b], 0.50[c]	0.19[b], 0.25[a,b]	0.25[a]	0.064[b], 0.13[a,c]	0.50[b,c]
Nonfermentative					
Achromobacter xylosoxidans	2.0[c], 4.0[a]	8.0[a]	8.0[a]	2.0[c], 4.0[a]	2.0[c]
Acinetobacter baumannii	0.03[a], 0.13[c]	0.06[a]	0.06[a]	0.13[a], 0.25[c]	0.25[c]
Acinetobacter calcoaceticus	0.016[a], 0.06[c]	0.016[a]	0.06[a]	0.13[a], 0.25[c]	0.25[c]
Acinetobacter johnsonii	0.016[a], 0.13[c]	0.016[a]	0.06[a]	0.06[a], 0.25[c]	0.50[c]
Acinetobacter junii	0.06[c]	-	-	0.25[c]	0.25[c]
Acinetobacter genospecies 3	0.016[a], 0.06[c]	0.016[a]	0.06[a]	0.13[a], 0.25[c]	0.25[c]
Chryseobacterium indologenes	0.25[c]	-	-	1.0[c]	1.0[c]
Chryseomonas luteola	0.13[c]	-	-	0.03[c]	0.13[c]
Stenotrophomonas maltophilia	0.13[a], 1.0[c]	0.25[a]	0.25[a]	0.50[a], 4.0[c]	4.0[c]
Pseudomonads					
Pseudomonas aeruginosa	0.50[b], 2.0[c], 4.0[a]	0.25[b], 4.0[a]	0.38[b], 2.0[a]	0.094[b], 0.25[c], 0.50[a]	0.75[b], 2.0[c]
Pseudomonas oryzihabitans	0.13[c]	-	-	0.03[c]	0.13[c]
Pseudomonas stutzeri	0.25[a,c]	0.13[a]	0.13[a]	0.03[a,c]	0.13[c]
Others					
Haemophilus influenzae	0.016[a], 0.03[c], 0.039[b,d]	0.008[a], 0.017[b,d]	0.024[b,d], 0.03[a]	0.008[a,c], 0.014[b,d]	0.03[c], 0.05[b,d]
Moraxella catarrhalis	0.03[a], 0.047[b,e], 0.06[c]	0.03[a,b,e]	0.016[a], 0.047[b,e]	0.016[a], 0.032[b,e,e]	0.13[b,e,e]
Moraxella osloensis	0.13[c]	-	-	0.13[c]	0.25[c]
Neisseria perflava	0.03[c]	-	-	0.008[c]	0.03[c]

[a] Published data from Bauernfeind et al.[7]
[b] Published data from Kowalski et al.[67]
[c] Unpublished data from Alcon.
[d] *Haemophilus* spp. rather than *Haemophilus influenzae.*
[e] *Moraxella* spp. rather than *Moraxella catarrhalis.*

hospital settings. With the recent market introduction of the fourth-generation fluoroquinolones (moxifloxacin and gatifloxacin) it is important to note that isolates resistant to second- and third-generation fluoroquinolones have decreased susceptibility to fourth-generation fluoroquinolones as well (Table 5). Ciprofloxacin-resistant isolates can be divided into two groups, those with moderate levels of resistance (2 to 8 μg/ml) and those with high-level resistance (16 μg/ml or higher). Very few isolates with moderate levels of resistance to ciprofloxacin were, in fact, resistant to fourth-generation fluoroquinolones. However, isolates that had a high-level resistance to ciprofloxacin were also classified as resistant to moxifloxacin. Table 6 presents recent data for ocular *S. aureus* and *S. epidermidis* isolates recovered from conjunctivitis from three different parts of the world. Based on the MIC_{90}s for moxifloxacin and ciprofloxacin, the 979 strains of *Staphylococcus aureus* and *S. epidermidis* are clearly more susceptible to moxifloxacin than ciprofloxacin throughout the three regions. Seppala et al reported *in vitro* resistance to moxifloxacin in up to 2.2% of *Streptococcus viridans* isolates from the normal flora of patients prior to cataract surgery.[102]

In spite of the increase in the number of fluoroquinolone-resistant pathogens recovered from ocular infections, there have not been reports of a corresponding increase in the number of treatment failures. In fact, it was recognized by NCCLS that its resistance breakpoint definition has no predictive value of topical therapy success or failure (**F2**).

TABLE 4

Susceptibility of Selected Atypical and Anaerobic Species to Five Fluoroquinolones

Bacterial Species	MIC_{50} (μg/ml)				
	Moxifloxacin	Gatifloxacin	Levofloxacin	Ciprofloxacin	Ofloxacin
Atypicals					
Mycobacterium avium	3.2[a]	6.4[a]	-	-	-
Mycobacterium marinum	0.4[a]	0.4[a]	-	-	-
Mycobacterium chelonae	1.6[a], 8.0[b]	1.6[a], 8.0[b]	32[b]	16[b]	-
Mycobacterium abcessus	8.0[b]	8.0[b]	16[b]	8.0[b]	-
Mycobacterium fortuitum group	0.06[b]	0.12[b]	0.25[b]	0.25[b]	-
Mycobacterium kansasii	0.06[d]		0.12[d]		
Chlamydia trachomatis	0.03[c]	-	0.25[c]	1.0[c]	1.0[c]
Anaerobes					
Propionibacterium acnes	0.25[c]	-	0.50[c]	0.50[c]	0.50[c]
Bacteroides fragilis	0.25[c]	-	1.0[c]	4.0[c]	2.0[c]
Clostridium perfringens	0.50[c]	-	0.50[c]	1.0[c]	1.0[c]
Peptostreptococcus spp.	0.25[c]	-	0.50[c]	0.50[c]	1.0[c]

[a] Published data from Saito et al.[96]
[b] Published data from Yang et al.[124]
[c] Published data from Fung-Tomc et al.[41]
[d] Published data from Alcaide et al.[2]

TABLE 5

Comparative Quinolone-Resistance in Ocular Isolates of Staphylococcus species[a]

Bacterial Species	Resistance Level	*n*	Moxifloxacin			Ciprofloxacin		
			MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
S. aureus	Moderate	20	0.016–2.0	0.13	2.0	2.0–8.0	2.0	8.0
	High	50	2.0–>32	4.0	8.0	16–≥128	128	≥128
S. epidermidis	Moderate	83	0.06–1.0	1.0	1.0	2.0–8.0	4.0	8.0
	High	48	0.50–>32	2.0	32	16–128	64	64
S. haemolyticus	Moderate	26	0.13–1.0	1.0	1.0	2.0–8.0	2.0	4.0
	High	13	1.0–8.0	2.0	4.0	16–≥128	16	≥128

[a] Unpublished data from Alcon.

TABLE 6

Geographic Differences in Fluoroquinolone Susceptibility in Staphylococcus Isolates Recovered from Conjunctivitis

Bacterial Species[a]	Region	*n*	Moxifloxacin			Ciprofloxacin		
			MIC Range	MIC_{50}	MIC_{90}	MIC Range	MIC_{50}	MIC_{90}
S. aureus[b]	USA	96	0.016–8.0	0.03	4.0	0.13–>128	0.25	64
	Europe	146	0.016–4.0	0.06	0.13	0.13–>128	0.25	1.0
	India	59	0.016–2.0	0.03	1.0	0.13–32	0.25	2.0
S. epidermidis	USA	335	0.03–>32	0.06	0.25	0.06–128	0.25	1.0
	Europe	216	0.03–32	0.06	0.13	0.06–64	0.25	0.50
	India	127	0.03–2.0	0.06	0.50	0.13–64	0.25	4.0
	Total	979						

[a] Unpublished data from Alcon (**F3**).
[b] Includes MRSA and MSSA.

Topically administered moxifloxacin ophthalmic solution 0.5% delivers high concentrations (i.e., 5,000 μg/ml) directly to the bacteria in superficial ocular infections. This concentration is 1,000 to 2,000 times that needed to inhibit growth of isolates classified as resistant *in vitro*. If the site of the ocular infection is deeper into the eye, the concentration of any topical antibiotic penetrating beyond the corneal

[F3] Stroman DW, Mendoza B, Sukplang P, et al: Kinetics of Killing of Ocular Isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* by Moxifloxacin. ARVO abstract #1463, 2003.

surface and into intraocular sites over time becomes critical. It may, in fact, be insufficient to inhibit the growth of a resistant organism in the posterior aspects of the eye.

MUTANT PREVENTION CONCENTRATION

A major concern with the use of any antibacterial agent is to what extent the use of the agent causes resistant strains to emerge. Although MICs give information about a specific antibiotic concentration that inhibits the growth of a bacterial strain, it does not distinguish between the bactericidal or bacteriostatic effects. A new term has been recently defined for fluoroquinolones is called mutant prevention concentration (MPC).[13,29]

Drlica reviewed this concept and proposed a "mutant selection window" which is an antimicrobial concentration range extending from the minimal inhibitory concentration required to block the growth of wild-type bacteria up to that required to inhibit the growth of the least susceptible, single-step mutant.[30] The upper boundary is the MPC. The MPC is often equivalent to the MIC of the most resistant mutant of a heterogeneous bacterial population. In general, the MPC will be 8 to 10-fold higher than the MIC for a reasonably susceptible isolate. An important technical difference between the two approaches is that MIC testing challenges the antibiotic with 10^5 colony forming units (CFUs), whereas MPC testing generally uses 10^{10} CFUs. The range of concentrations between MIC and MPC is defined as the mutant selection window.[32,127] Considerable efforts are being made to define the relationship between pharmacodynamic properties of the antibacterial agent and the mutant-selection window.[5,21] The length of time to maintain the antibacterial agent concentration above the MPC at the site of infection and its impact on emergence of resistant strains has yet to be clinically established. The MPC is an important concept in preventing the growth of organisms that have some level of drug resistance prior to therapy. The concentration of antibiotic must be maintained above the MPC to resist the selection of mutants that would give rise to a population of organisms that are not susceptible to the antibiotic.

KINETICS OF KILL TESTING

Kinetics of kill studies are *in vitro* tests that assess the rate of killing by a particular antibiotic of specific bacterial isolates. For technical reasons, testing is almost always performed at a constant concentration of antibiotic throughout the entire time of exposure of drug to the logarithmically growing bacteria. This *in vitro* assay has been useful in distinguishing the bacteriostatic and bactericidal effects of many antibiotics. Kinetics of kill studies have been performed with antibiotic concentrations equal to multiples of the MIC; for example, 2X MIC, 4X MIC, 8X MIC, 10X MIC, and so forth. Moxifloxacin is rapidly bactericidal in such studies.[16] Another testing approach uses dilutions of antibiotic products. For example, in superficial infections such as conjunctivitis, testing has been performed at 1:10 and 1:100 dilutions of the product to represent the tear film concentrations at 10 and 30 minutes after topical dosing.

Staphylococci represent the most frequently isolated group of bacteria from superficial ocular infections (e.g., conjunctivitis and blepharitis), deeper ocular infections (e.g., endophthalmitis), as well as from the conjunctiva and skin/lid lash margins of healthy eyes. The kinetics of kill by moxifloxacin of *S. aureus* and *S. epidermidis* (quinolone-susceptible and quinolone-resistant) isolates, was recently reported (**F3**). As shown in Figs. 1 and 2, moxifloxacin, at 500 µg/ml and 50 µg/ml, kills faster than ciprofloxacin at 300 and 30 µg/ml. These concentrations were tested as they correspond to 1:10 and 1:100 dilutions of the formulated products. Nevertheless, the faster kill by moxifloxacin compared to ciprofloxacin makes it an important option when rapid eradication of organisms on or near the surface of the eye is warranted. Rapid eradication minimizes the amplification of subpopulations of resistant strains.[121,127]

In Vivo Potency

ASSESSMENT OF FLUOROQUINOLONE THERAPY IN RABBIT KERATITIS MODELS

Several reports have established the effectiveness of fluoroquinolones in the treatment of experimental keratitis. Data from Barequet et al have shown the improved efficacy of a third-generation fluoroquinolone (trovafloxacin) as compared to ciprofloxacin and ofloxacin in a rabbit model of *S. aureus* and *P. aeruginosa* keratitis.[6] Their results showed that the third-generation fluoroquinolone had approximately a 2-log greater reduction in CFU/cornea relative to the second-generation fluoroquinolone in treating *S. aureus* and all the fluoroquinolones studied sterilized the corneas in their *P. aeruginosa* model.[6]

Furthermore, other studies using a rabbit model of keratitis induced by corneal injection of *P. aeruginosa* demonstrated that there were no significant differences between moxifloxacin and ciprofloxacin in the treatment of *P. aeruginosa*.[4]

The *in vivo* efficacies of commercial solutions of 0.5% moxifloxacin (VIGAMOX®, Alcon, Fort Worth,

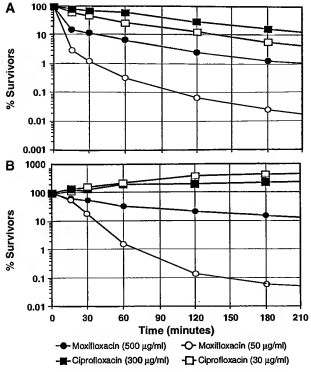

Fig. 1. Kinetics of kill of *Staphylococcus aureus*. *A:* Quinolone-sensitive (ciprofloxacin MIC = 0.25 µg/ml) *S. aureus* were incubated with moxifloxacin or ciprofloxacin and the percent survivors were determined at various time points. *B:* Quinolone-resistant (ciprofloxacin MIC = 128 µg/ml) *S. aureus* were incubated with moxifloxacin or ciprofloxacin and the percent survivors were determined at various time points.

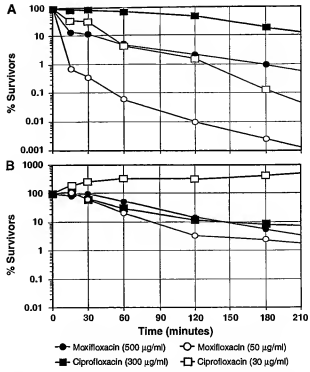

Fig. 2. Kinetics of kill of *Staphylococcus epidermidis*. *A:* Quinolone-sensitive (ciprofloxacin MIC = 0.13 µg/ml) *S. epidermidis* were incubated with moxifloxacin or ciprofloxacin and the percent survivors were determined at various time points. *B:* Quinolone-resistant (ciprofloxacin MIC = 64 µg/ml) *S. epidermidis* were incubated with moxifloxacin or ciprofloxacin and the percent survivors were determined at various time points.

TX), 0.3% levofloxacin (Quixin®), and 0.3% ciprofloxacin (Ciloxan®) were compared in the treatment of experimental keratitis caused by *S. aureus* (with various sensitivities to methicillin and second-generation fluoroquinolones),[24] *P. aeruginosa*, and *Serratia marcescens* keratitis in rabbits.[112] Two treatment models were used with the *S. aureus*: an early treatment model where bacteria were actively replicating and a late treatment model where bacteria were in their stationary phase of growth. *P. aeruginosa* and *S. marcescens* keratitis models involved treatment of bacteria in their slow replication phase, but not yet in their stationary phase. The results from these animal studies of ocular infections treated with fluoroquinolones are shown in Figs. 3–6 and reviewed below.

TREATMENT OF FLUOROQUINOLONE-SENSITIVE *STAPHYLOCOCCUS AUREUS* KERATITIS

Early treatment of rabbit eyes infected with ofloxacin-sensitive MSSA or MRSA demonstrated that moxifloxacin, levofloxacin, or ciprofloxacin reduced the number of *S. aureus* equally by approximately 5-log CFU/cornea as compared to the untreated control group (Figs. 3A and 3B).[24] Eyes with established infections caused by bacteria that are not actively replicating are more refractory to antibiotics. Therefore, moxifloxacin, levofloxacin, and ciprofloxacin were evaluated to determine their effectiveness in treating such infections. Late treatment of infected rabbit eyes with moxifloxacin, levofloxacin, or ciprofloxacin produced approximately 5, 4, or 2.5-log reduction in CFU/cornea, respectively, relative to the control group (Figs. 3C and 3D).[24] None of the fluoroquinolones tested were as effective as moxifloxacin in reducing *S. aureus* in the late treatment model. Moxifloxacin was shown to be the most effective therapy demonstrating its activity in both the early and later treatment schedules. *S. aureus* produce tissue destructive exoproteins as the growth of the organisms goes from an actively replicating state (log phase) to a stationary phase of growth. During the stationary phase is where inflammation occurs and is the point when patients seek medical attention.

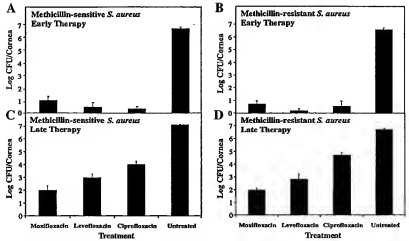

Fig. 3. Early and late fluoroquinolone treatment of ofloxacin-sensitive *Staphylococcus aureus* keratitis in rabbits. Early (*A* and *B*) and late (*C* and *D*) fluoroquinolone therapy of rabbit eyes infected with MSSA or MRSA sensitive to ofloxacin (MIC = 0.5 μg/ml). (Reprinted from Dajcs et al[24] with permission of the American Society for Microbiology.)

TREATMENT OF FLUOROQUINOLONE-RESISTANT *STAPHYLOCOCCUS AUREUS* KERATITIS

Early treatment of rabbit eyes infected with ofloxacin-resistant MSSA or MRSA with moxifloxacin, levofloxacin, or ciprofloxacin produced approximately 4.5, 3.5, or 0.5-log reductions in CFU/cornea, respectively, relative to the untreated eyes (Figs. 4A and 4B).[24]

Late treatment of the infected rabbit eyes with either levofloxacin or ciprofloxacin did not produce significant reductions in CFU relative to the untreated control (Figs. 4C and 4D).[24] During late treatment, only moxifloxacin was able to significantly reduce the CFU/cornea as compared to the untreated group.

TREATMENT OF *PSEUDOMONAS AERUGINOSA* KERATITIS

A study by Rhee et al employed a rabbit keratitis model to demonstrate that ciprofloxacin-sensitive *P. aeruginosa* (MIC = 2) can be effectively treated with ciprofloxacin.[94] Additionally, they showed that ciprofloxacin-resistant *P. aeruginosa* (MIC > 32) was not effectively treated with ciprofloxacin.[93] Treatment of *P. aeruginosa* keratitis with moxifloxacin, levofloxacin, ciprofloxacin, or ofloxacin resulted in a 5

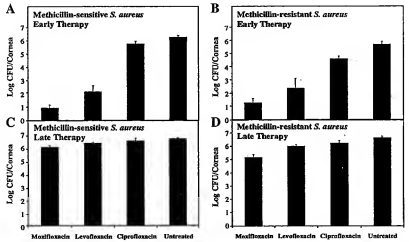

Fig. 4. Early and late fluoroquinolone treatment of ofloxacin-resistant *Staphylococcus aureus* keratitis in rabbits. Early (*A* and *B*) and late (*C* and *D*) fluoroquinolone therapy of rabbit eyes infected with MSSA or MRSA resistant to ofloxacin (MIC = 128 μg/ml). (Reprinted from Dajcs et al[24] with permission of the American Society for Microbiology.)

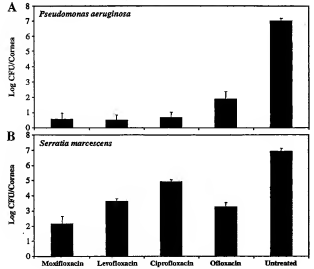

Fig. 5. Fluoroquinolone treatment of *P. aeruginosa* or *S. marcescens* keratitis in rabbits. *A:* Fluoroquinolone therapy of rabbit eyes infected with ciprofloxacin-sensitive *P. aeruginosa* (MIC = 0.13 μg/ml). *B:* Fluoroquinolone therapy of rabbit eyes infected with ciprofloxacin-sensitive *S. marcescens* (MIC = 0.13 μg/ml). (Figure adapted from Thibodeaux BA et al.[112])

or greater log reduction in CFU/cornea as compared to the untreated group (Fig. 5).[112] These data demonstrate that moxifloxacin is equal to or more effective in treating infections caused by *P. aeruginosa* then the second- and third-generation fluoroquinolones like ciprofloxacin or levofloxacin.

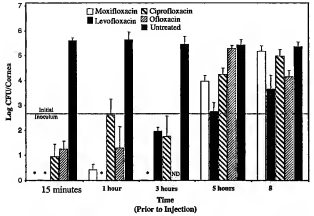

Fig. 6. Fluoroquinolone prophylaxis of experimental keratitis caused by *Staphylococcus aureus.* Rabbit eyes were treated with a single topical application of fluoroquinolone at various time points prior to inoculation with approximately 500 CFU of *S. aureus* (ciprofloxacin MIC = 0.5 μg/ml). * - Sterile eyes; ND - not determined. (Based on data from Dajcs et al.[22,23])

TREATMENT OF *SERRATIA MARCESCENS* KERATITIS

Treatment of *S. marcescens* keratitis with moxifloxacin, levofloxacin, ciprofloxacin, or ofloxacin produced approximately a 5, 3, 2, 3.5-log reduction in CFU/cornea, respectively, relative to the untreated control (Fig. 5).[112] Moxifloxacin was the most effective therapy in the reduction in CFUs of *S. marcescens* in the cornea.

PROPHYLAXIS OF EXPERIMENTAL *STAPHYLOCOCCUS* KERATITIS

Recent reports have shown that the occurrence of postsurgical infections following ocular surgery is increasing.[20,61,84] Although other organisms, like *Streptococcus* can cause endophthalmitis,[80] the most common organisms isolated in postsurgical endophthalmitis are *S. epidermidis* and *S. aureus*[48,105] and are derived mainly from the patient's own eyelids.[47,105] Given the ability of surface microbial flora to enter the eye during surgery, prophylactic antibiotics have been administered before, during and after ocular surgery to reduce the risk of postsurgical infections, especially keratitis or endophthalmitis.[8,70]

In order to prevent postoperative ocular infections, a drug must reach the relevant tissues at appropriate concentrations. Topical ophthalmic preparation must overcome tear dilution, mechanical action of the eyelids, and rapid run off over the concave corneal surface. Antimicrobial agents with good ocular penetration in the tear film, cornea, and aqueous humor are needed to prevent post-surgical ocular infections.[86] Topical antibiotics may reduce the risk of post-surgical infection by sterilizing the ocular surface, preventing the introduction of bacteria into the eye by maintaining a lethal concentration in ocular tissue, thereby killing any exogenous bacteria introduced during surgery.

Ophthalmologists have been using fluoroquinolones for prophylactic treatment to reduce the risk of ocular infections for many years. Fluoroquinolones have been used prophylactically prior to ocular surgery because of their broad-spectrum of coverage and good penetration into tissues and aqueous humor.[77,79] Ciprofloxacin and ofloxacin[18,49,123] and levofloxacin[95] have been shown to achieve significant intraocular concentrations. Furthermore, fourth-generation fluoroquinolones, such as moxifloxacin and gatifloxacin, have been shown to achieve higher concentrations in the aqueous humor than older fluoroquinolones.[50,72,79] (F4) Levine et al demonstrated that the topical application of moxifloxacin produced approximately a 45% higher concentration in the aqueous humor when comparison to gatifloxacin.[72] Hariprasad et al found that topical administration of moxifloxacin ophthalmic solution

0.5% every 2 or 6 hours for 3 days before surgery resulted in antibiotic concentrations of 2.28 μg/ml in the aqueous humor and 0.11 μg/ml in the vitreous in 2-hour group and 0.88 μg/ml (aqueous) and 0.06 μg/ml (vitreous) in the 6-hour group.[50] There is no consensus as to how frequently an antibiotic should be administered for adequate prophylaxis. In Miller's review of eye infections, corneal concentrations achievable by topical administration of fluoroquinolones ranged from 0.04 to 5.29 μg/ml.[81] Robertson et al reported ocular penetration of moxifloxacin up to 24.8 μg/g cornea, which was five times greater than that for gatifloxacin (4.85 μg/ml) after topical administration in rabbits.[22] Other investigators saw a two-fold increase of moxifloxacin over gatifloxacin in the aqueous humor of humans (**F4**).[104] A recent study compared timing and frequency of topical ofloxacin administered 1 hour or 3 days prior to surgery.[107] Additional studies have indicated a reduction in positive conjunctival cultures both immediately before and after surgery, signifying the importance of prophylactic topical antibiotic therapy.[86,107]

Several studies have evaluated second-generation fluoroquinolones (i.e., ciprofloxacin and ofloxacin) as prophylactic antibiotics for the prevention of experimental *S. aureus* keratitis.[22,23,115] Another study showed the effectiveness of a fourth-generation fluoroquinolone (gatifloxacin) in the prevention of multi-drug-resistant *S. aureus* keratitis after lamellar keratectomy in a rabbit model.[115] The fluoroquinolones were quantitatively evaluated for their effectiveness in preventing growth of the *S. aureus* inoculum by applying fluoroquinolone at various times prior to inoculation of bacteria and enumerating viable cell counts.

Data presented in Fig. 6 show a comparison of prophylactic effectiveness of moxifloxacin, levofloxacin, and two second-generation fluoroquinolones. All fluoroquinolones when applied 3 hours or less prior to inoculation prevented an increase in the number of bacteria relative to the initial inoculum (500 CFU); however, only moxifloxacin sterilized all the eyes. None of the fluoroquinolones were effective in reducing the CFU relative to the inoculum when applied at 5 or 8 hours prior to infection. In this prophylaxis model of keratitis, topical moxifloxacin demonstrated superior penetration across an intact corneal epithelium and to a lethal concentration, for up to 3 hours in the cornea and anterior chamber at concentrations that were lethal to the organisms at the time of injection.

[F4] McCulley JP, Surratt G, Shine W: 4th generation fluoroquinolone penetration into aqueous humor in humans. Invest Ophthalmol Vis Sci 45: Abstract 4927, 2004.

Topical moxifloxacin applied pre-challenge, post-challenge, or pre-and post-challenge has been demonstrated to prevent endophthalmitis with an inoculum of 50,000 CFU of *S. aureus* into the anterior chamber.[69] This is the first report demonstrating that topical application of antibiotic can prevent experimental endophthalmitis.

Tungsiripat et al have also demonstrated the improved effectiveness of a fourth-generation fluoroquinolones in relation to second and third-generation fluoroquinolones for the prevention of multiple-drug-resistant *S. aureus* keratitis after lamellar keratectomy.[115] Their data showed that ciprofloxacin or levofloxacin were 45% effective in preventing keratitis, whereas, gatifloxacin was 100% effective in preventing multiple drug-resistant *S. aureus* keratitis. These experiments have established that fourth-generation fluoroquinolones can be successful prophylactic antibiotic for the prevention of keratitis or endophthalmitis. According to Thauvin-Eliopoulos, it is both the broad-spectrum antimicrobial activity of these drugs, and their high potency against many susceptible strains, that position the fluoroquinolone antimicrobials among our most valuable therapeutic classes.[111]

Conclusions

In vitro studies have shown that moxifloxacin has improved activity for gram-positive and atypical organisms and similar activity against gram-negative organisms compared to second and third-generation fluoroquinolones (i.e., ofloxacin, ciprofloxacin, levofloxacin). Moxifloxacin inhibited the growth of bacteria frequently isolated from ocular infections and had a faster rate of killing of fluoroquinolone-resistant organisms than ciprofloxacin. Furthermore, *in vivo* studies demonstrated that moxifloxacin ophthalmic solution 0.5% is an effective topical therapy for treatment or prevention of experimental bacterial keratitis. Moxifloxacin penetrates anterior ocular tissues (e.g., cornea, tear film, conjunctiva, iris-ciliary body, aqueous humor) to concentrations at or above the MIC of the major ocular pathogens.[22,62] The high intraocular concentrations achieved coupled with the inate *in vitro* and *in vivo* potency, as well as the inhibition of the bacterial efflux mechanism enhances the ability of moxifloxacin to accumulate to lethal levels at the infected site and thereby minimize the rate of resistance to the drug.

Method of Literature Search

A literature search for this article was performed based on MEDLINE database searches from 1966 to 2005, using varying combinations of the search terms

ocular infections, fluoroquinolones, generations, mechanism of action, mutant prevention concentration, ocular penetration, prophylaxis, animal models of keratitis, keratitis, endophthalmitis, and resistance. Relevant journal articles were selected for review. Articles cited in the references of journal articles were also included. An effort to use the most recently available literature was made, concentrating on journal articles published in the last decade.

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Ocular Pharmacokinetics of Moxifloxacin After Topical Treatment of Animals and Humans

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Abstract. The ocular penetration and pharmacokinetics of moxifloxacin in comparison to other fluoroquinolones (ofloxacin, ciprofloxacin, gatifloxacin, norfloxacin, levofloxacin, and lomefloxacin) have been determined by *in vitro* and *ex vivo* techniques, as well as in animal and human studies. This article reviews the original pharmacokinetics work performed by Alcon and other studies reported in the ocular fluoroquinolone literature. The results consistently demonstrate higher maximum concentrations for moxifloxacin relative to the other fluoroquinolones in ocular tissues with levels well above its minimum inhibitory concentrations for relevant ocular pathogens. This superior performance is due to the unique structure of moxifloxacin that combines high lipophilicity for enhanced corneal penetration with high aqueous solubility at physiological pH. The latter property creates a high concentration gradient at the tear film/corneal epithelial interface providing a driving force for better ocular penetration for moxifloxacin. In addition, the higher concentration of moxifloxacin in VIGAMOX® (i.e., 0.5% vs. 0.3%) allows more antibiotic to be available to ocular tissues. It is clear from the array of studies summarized in this report that moxifloxacin penetrates ocular tissues better (two- to three-fold) than gatifloxacin, ciprofloxacin, ofloxacin, or levofloxacin. This consistent, enhanced penetration of topical moxifloxacin offers powerful advantages for ophthalmic therapy. (Surv Ophthalmol 50:S32–S45, 2005. © 2005 Elsevier Inc. All rights reserved.)

Key words. fluoroquinolones • moxifloxacin • ocular penetration • ophthalmic therapy • pharmacokinetics • VIGAMOX®

Introduction

Moxifloxacin is a novel fourth-generation fluoroquinolone with high potency against both gram-positive and gram-negative bacterial pathogens. It has the highest potency in its class against *Staphylococcus aureus* and *Staphylococcus epidermidis*.[10] Moxifloxacin has been developed as a 0.5% solution for topical, ocular use as moxifloxacin ophthalmic solution 0.5%, (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX). In addition to high potency, a desirable characteristic of topical antibiotics is the rapid migration across the cornea and extensive penetration into anterior ocular tissues. The ocular bioavailability of different antibiotics can be compared on the basis of the concentration achieved in the tears, cornea, conjunctiva, aqueous, or vitreous humor. The concentration of the antibiotic in these tissues should be maintained for sufficient time above the minimum

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inhibitory concentration (MIC) for important pathogens in order to achieve effective bacterial killing. The higher the concentration above the antibiotic's MIC, the greater the protection against infection. Alcon and other investigators have conducted numerous non-clinical and clinical studies to measure the ocular uptake and pharmacokinetics (PK) of moxifloxacin in comparison to other topical fluoroquinolones. The results of these recent *in vitro, ex vivo, in vivo,* and human clinical studies are reviewed in this article.

Materials and Methods

IN VITRO/EX VIVO METHODS

For most of the *in vitro* or *ex vivo* corneal penetration experiments, fresh, excised corneas from rabbits were mounted in corneal perfusion chambers. Solutions of fluoroquinolones or commercial products were applied to the epithelial side of the excised corneas for a period of time (e.g., 5 minutes) and the amount of antibiotic crossing the cornea was measured on the endothelial side. Dembinska and colleagues (F1) measured the effects of topical antibiotic preparations on the corneal epithelial barrier function and also measured the rate of carboxyfluorescein or sodium fluorescein permeation through the treated excised corneas. In this study, the permeation of each dye was assessed by linear regression fitted to the ascending part of its curves. Statistical analysis was performed on individual slopes ($n \geq 4$) and included the mean, the standard deviation, and one-way ANOVA and Tukey tests for multiple comparisons. Each analysis included controls pooled from both fluoroquinolones. In other *in vitro* studies canine kidney (MDCK) cells were used as a model to assess and compare tissue penetration properties for various fluoroquinolone formulations (F2). For these studies, linear regressions were determined using S-Plus 6.0 for Windows, Release 2, from the Insightful Corporation.

IN VIVO ANIMAL STUDIES

For *in vivo* rabbit studies, animals were treated topically with various fluoroquinolone formulations and euthanized at the appropriate time-points following topical treatment. The ocular tissues were analyzed for fluoroquinolone content. Aqueous humor, vitreous humor, tear film, and plasma samples were analyzed using aliquots of fixed volume while other tissues (i.e., cornea, iris-ciliary body, conjunctiva) were weighed and homogenized in water prior to analysis.

HUMAN CLINICAL STUDIES

For human studies, aqueous and vitreous humor samples were obtained using a syringe and canula during cataract or other ophthalmic surgeries. Also, samples of conjunctiva were excised from normal human volunteers after topical instillation of fluoroquinolone preparations[15] (F3).

ANALYSIS OF SAMPLES

For all studies, tissue samples were generally stored in sealed vials at −20°C or colder until analyzed. Concentrations of moxifloxacin and other fluoroquinolones were determined using high performance liquid chromatography (HPLC) with fluorescence detection or HPLC/tandem mass spectrometry. Concentrations were normally given as μg/ml or μg/g of tissue. Lower limits of quantitation ranged from 2–30 ng/g or ng/mL depending on the ocular tissue.

DOSING REGIMENS

Dosing regimens for animal and human studies varied according to each protocol. The dosing details of the *in vivo* and clinical phases of various studies are discussed in the sections below or given in the Tables.

Results

IN VITRO/EX VIVO STUDIES

The corneal penetration and permeability characteristics of moxifloxacin were investigated in a series of Alcon *in vitro* and *ex vivo* studies that are summarized below.

Ocular *Ex Vivo* Penetration and Corneal Permeability of Moxifloxacin and Gatifloxacin

One Alcon study (F4) involved exposing the epithelial side of excised corneas from New Zealand white rabbits to 0.004% (0.1 mM) solutions of moxifloxacin and gatifloxacin in BSS balanced salt solution. The concentrations of the fluoroquinolones were determined by HPLC of the perfusates over a 5-hour period. The results in Table 1 show a 3.6-fold

[F1] Dembinska O, Stout KR, Podval J, Rodeheaver DP: Corneal epithelial barrier function following the exposure to VIGAMOX® and Zymar in *ex vivo* model of corneal penetration. Invest Ophthalmol Vis Sci 46: E-Abstract 4901, 2005.

[F2] Rusinko A, May J, Liao J, Namil A, Hellberg M: A study of the enhanced corneal penetration of moxifloxacin. Invest Ophthalmol Vis Sci 45: E-Abstract 4907, 2004.

[F3] Wagner RS, Abelson MB, Shapiro A, Torkildsen G: Evaluation of fluoroquinolone antibiotic concentrations in human conjunctival tissue following topical administration. Invest Ophthalmol Vis Sci 46: E-Abstract 4888, 2005.

TABLE 1

In Vitro/Ex Vivo Penetration Study for Moxifloxacin and Gatifloxacin (F4)

	Moxifloxacin*	Gatifloxacin*	Difference
Corneal permeability to FQ ($\times 10^{-9}$ cm/sec ± SD)	91 ± 9	25 ± 2	moxifloxacin penetrated 3.6 × more than gatifloxacin (P = 0.005)**
Time to appearance of FQ on endothelial side of cornea (min ± SD)	49 ± 1	99 ± 12	moxifloxacin penetrated 2.0 × faster than gatifloxacin (P = 0.02)**

* Test articles were 0.004% solutions of fluoroquinolone in a preservative-free vehicle and each mean value was the average of three corneas ± Standard deviation.

** Significance determined with paired t-test.

better penetration (i.e., higher corneal permeability) for moxifloxacin versus gatifloxacin. In addition, the time before the appearance of antibiotic on the endothelial side was about two-fold earlier (i.e., quicker penetration) for moxifloxacin than for gatifloxacin. In this study, none of these solutions contained benzalkonium chloride (BAC) as a preservative.

Another Alcon study (F1, F4) used two commercial antibiotic products and determined the corneal permeability to carboxyfluorescein (CF) or sodium fluorescein (SF) as a measure of corneal integrity. In this *ex vivo* permeability study, the commercial fluoroquinolone preparations (VIGAMOX® and Zymar® [gatifloxacin ophthalmic solution 0.3%; Allergan, Inc, Irvine, CA]) were applied to the epithelial surface of the excised cornea of the rabbit for 5 minutes. After rinsing, corneas were exposed to either CF or SF for 5 minutes and the perfusate collected over 2 hours. The level of CF or SF in the perfusate was measured by spectrophotometry and the more CF or SF found in the perfusate then the more corneal damage occurred. The results in Table 2 show again that moxifloxacin penetrates the cornea better than gatifloxacin (6.5 vs 2.8 μg/min) and faster (11 vs. 18 min). Also, CF and SF passed through the cornea faster after treatment with Zymar than after treatment with VIGAMOX® (2.77 vs 1.83 pMol/ml/min for CF, 0.50 vs. 0.28 pMol/ml/min for SF). Although moxifloxacin penetrates the cornea faster and accumulates at higher concentrations in the aqueous humor than gatifloxacin (Table 1 and 2), VIGAMOX® maintained the corneal integrity better than Zymar®. There was a 1.6-fold lower accumulation of CF (37 vs. 60 pMol/ml) after treatment with VIGAMOX® than with Zymar® (Table 2). The presence of benzalkonium chloride in Zymar is likely causing a loss of integrity to the intercellular (gap) junctions in the corneal epithelium, whereas VIGAMOX® has an advantage in this regard (i.e., it is self-preserved and contains no benzalkonium chloride) (F4). These results indicate that the enhanced corneal penetration of moxifloxacin relative to gatifloxacin is due to inherent differences in molecular structure, the higher fluoroquinolone concentration (0.5% vs. 0.3%), and the lack of BAC in the commercial preparation.

Lipophilicity and MDCK Cell Permeability of Fluoroquinolones (Fig. 1)

Lipophilicity and aqueous solubility are two factors that govern corneal penetration rates. The purpose of this Alcon study (F2) was to identify which molecular properties are responsible for the superior bioavailability of topically applied moxifloxacin in comparison to six other fluoroquinolones. Secondly, a mathematical model was developed to predict corneal permeability of fluoroquinolone antibiotics based upon their *in vitro* data using Madin-Darby canine kidney (MDCK) cells and physiochemical properties. For the MDCK studies, 10 μM solutions of fluoroquinolones were tested. Aqueous solubility and lipophilicity values were determined for each fluoroquinolone. The permeabilities of seven fluoroquinolones (i.e., moxifloxacin, ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, lomefloxacin, and gatifloxacin) were determined in the MDCK model and the *in vitro* corneal penetration data published earlier by Fukada.[5] The results of these comparisons are shown in Table 3. The MDCK permeability showed a high correlation with lipophilicity (R^2 = 0.92 see Fig. 1) and corneal permeability (R^2 = 0.93) indicating that the *in vitro* MDCK model is an excellent predictor of corneal penetration. Moxifloxacin had the highest molecular weight (401.4) and showed higher lipophilicity (0.24), greater aqueous solubility (>6.43%) and the best MDCK and corneal

[F4] Owen GR, Dembinska O, Stout KR, Mendiola MK: Corneal penetration and changes in corneal permeability of moxifloxacin versus gatifloxacin. Invest Ophthalmol Vis Sci 45: E-Abstract 4910, 2004.

TABLE 2.

In Vitro/Ex Vivo Permeability Studies with Two Commercial Topical Fluoroquinolones (F1, F4)

	Moxifloxacin* mean ± SD	Gatifloxacin* mean ± SD	Control (Balanced Salt Solution) mean ± SD	Difference**
Rate of accumulation of FQ in the cornea; value in μg/min	6.5 ± 1.6	2.8 ± 0.5	Not Applicable	moxifloxacin penetrated 2.3 × more than gatifloxacin
Lag time before FQ appearance in the cornea; value in minutes	11 ± 4	18 ± 5	Not Applicable	moxifloxacin was 1.6 × faster in getting to the cornea
Rate of carboxyfluorescein (CF) permeation (slope in pMol/mL/min)	1.83 ± 0.68	2.77 ± 1.19	0.71 ± 0.83	Zymar caused CF to penetrate 4 × more than control ($P < 0.001$) and 1.5 × more than VIGAMOX® ($P < 0.05$)
Rate of sodium fluorescein (SF) permeation (slope in pMol/mL/min)	0.28 ± 0.13	0.50 ± 0.17	0.85 ± 0.16	Zymar caused SF to penetrate 1.8 × more than VIGAMOX® ($P < 0.05$)
Peak carboxyfluorescein (CF) accumulation (pMol/mL)	37 ± 9	60 ± 5	18 ± 8	Zymar caused CF to penetrate 1.6 × more than VIGAMOX® ($P < 0.05$)

* Test articles were the commercial preparation of moxifloxacin 0.5% (VIGAMOX®) and gatifloxacin 0.5% (Zymar®). Each mean value was the average of four corneas ± standard deviation.

** Significance determined with paired *t*-test where indicated.

*** Minutes as $\log_e$ scale.

permeability (35.2 and 15.8, respectively) than any of the other six fluoroquinolones tested (F2).

IN VIVO ANIMAL STUDIES

Numerous *in vivo* studies conducted in rabbits have characterized the ocular penetration and pharmacokinetics of moxifloxacin and other fluoroquinolones as comparators.

Ocular Penetration of 0.3% Solutions of Moxifloxacin and Ofloxacin

This Alcon study (F5) was designed to measure the ocular penetration and distribution of moxifloxacin and ofloxacin into the aqueous humor, cornea, iris-ciliary body, tear film, and plasma following a single topical ocular administration of 0.3% solutions to rabbits. The eyes of male Dutch-belted rabbits were dosed with a single drop (30μl) of either 0.3% moxifloxacin or 0.3% ofloxacin solutions. The moxifloxacin 0.3% solution tested was prepared as a 0.3% solution in phosphate buffer saline (pH 7.4); the commercial product (VIGAMOX®) contains a higher concentration of moxifloxacin, namely, 0.5%. The 0.3% ofloxacin solution was the commercially available Ocuflox® (pH 6.4; Allergan, Irvine, CA). Aqueous humor, cornea, iris-ciliary body, tear film, and plasma were collected up to 48 hours post-dose for analyses of drug concentrations by reverse phase HPLC. The corneas from the animals were not altered in any way to increase absorption. The investigators were careful to ensure the integrity of the corneal epithelium in the study. The in-life work was conducted at a high-quality independent GLP research laboratory. All animals underwent an examination with sodium fluorescein prior to dosing to verify the integrity of the cornea. This examination was documented for every eye in the study. The study directions specified that extreme care be taken to avoid any injury to the corneas in all groups throughout the study. The moxifloxacin and ofloxacin concentrations achieved are given for the various times post-dose in Table 4 for aqueous humor, cornea, iris-ciliary body and plasma. The moxifloxacin concentrations were typically higher than ofloxacin in all tissues tested and at most time pulls. The maximum concentration (Cmax) for moxifloxacin was two-fold higher than ofloxacin in the cornea and 3.5-fold higher in the aqueous humor. Moxifloxacin's Cmax for iris-ciliary body and plasma were 2.5- and 1.5-fold

[F5] Robertson SM, Sanders M, Jasheway D, Trawick D, Veltman J, Hamner S, Schlech BA, Hilaski R, Dahlin DC: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits. Invest Ophthalmol Vis Sci 44: E-Abstract 1454, 2003.

TABLE 3

Molecular Properties of Fluoroquinolones (F2)

Fluoroquinolone	Molecular Weight	Aqueous solubility (%)	MDCK Permeability (cm/s) × 10^7	Corneal Permeability* (cm/s) × 10^7	Lipophilicity C-7, π
Moxifloxacin	401.4	>6.43**	35.2	15.8	0.24
Gatifloxacin	375.4	0.21	10.3	4.6	0.11
Ciprofloxacin	331.3	0.02	4.5	2.46	−0.35
Ofloxacin	361.4	0.35	15.1	6.78	0.06
Norfloxacin	319.3	0.05	3.3	1.65	−0.35
Levofloxacin	361.4	1.85**	16.4	6.95	0.06
Lomefloxacin	351.3	0.13	6.6	3.58	0.11

* Data for moxifloxacin and gatifloxacin were estimated by linear regression; Data for remaining five fluoroquinolones were from Fukada and Sasaki.[5]

** After 13 weeks of mixing.

higher than ofloxacin's Cmax. Ofloxacin concentrations in the cornea fell below its MIC_{90} of 0.5 μg/ml for methicillin-resistant *Staphylococcus aureus* (MRSA) by 8 hours while the moxifloxacin concentration (0.25 μg/ml) was about four-fold higher than its MIC_{90} (0.06 μg/ml) for MRSA even at 48 hours after instillation. In aqueous humor, ofloxacin concentrations fell below the quantitation limit of detection (i.e., 33 ng/g) after 4 hours, whereas there were still measurable amounts of moxifloxacin found at the last time point of 48 hours. The moxifloxacin iris-ciliary body concentrations were substantially higher than those for ofloxacin. These results demonstrate that moxifloxacin has a better penetration profile compared to ofloxacin. Results from other Alcon studies (unpublished Alcon data) indicate that ofloxacin has a better penetration profile than ciprofloxacin. Following single-drop instillation of 0.3% ciprofloxacin solution, the mean ciprofloxacin concentration at 1 hour was 7.3-fold lower than that for ofloxacin (0.076 μg/g vs. 0.555 μg/g, respectively). Based on the results of these moxifloxacin, ofloxacin, and ciprofloxacin single-drop studies, it can be concluded that the penetration profile of moxifloxacin into the aqueous humor and cornea is greater than ofloxacin, which is greater than that for ciprofloxacin. Also maximal plasma concentrations of both moxifloxacin and ofloxacin were low (<0.01 μg/ml) and declined rapidly in the plasma. The moxifloxacin plasma concentration in this study was 700-fold lower than the tolerated doses from toxicology studies and 300-fold lower than those measured in clinical studies at oral doses. Even at these high oral doses, there were no relevant deviations from normal biochemical or hematological parameters. These results demonstrate a wide margin of safety for topical ophthalmic use of 0.3% moxifloxacin solutions.

Tear Film Concentrations of Moxifloxacin and Ofloxacin (Fig. 2)

In this Alcon study (F5), nine male Dutch-Belted rabbits were used to measure the tear film concentrations achieved after a single drop of 0.3% moxifloxacin or 0.3% ofloxacin solutions. Each animal, except the undosed controls (n = 3/group), received a single 30-μl topical ocular dose to right (OD) eye using a calibrated positive displacement pipettor. Tear film samples (0.5–1.0 μl) were collected from the dosed eyes using capillary tubes at 1, 2, 3, 5, 60, 90, 120, 180, 240, and 360 minutes. Samples were weighed and stored at −70°C prior to analysis by reverse phase HPLC. The results of this single-drop study are shown in Fig. 2 and indicate that at the early time pulls, the concentrations were similar, but over the course of the next hour, ofloxacin concentration dropped more rapidly than moxifloxacin with moxifloxacin levels being about seven-fold to 10-fold higher than ofloxacin. Ofloxacin was not detectable in the tear film (i.e., <0.5 μg/ml) beyond 90 minutes whereas moxifloxacin remained at concentrations at or above 1.0 μg/ml up to the last time point of 6 hours.

Effect of Cataract Surgery on Ocular Levels of Moxifloxacin

Another study by Mather and colleagues[12] at the Proctor Foundation mimicked cataract surgery in Dutch-belted rabbits. The moxifloxacin concentrations in the aqueous and vitreous humors were measured 30, 60, and 120 minutes after topical instillation of moxifloxacin ophthalmic solution 0.5%. Mean tissue concentrations obtained in surgical eyes were compared with concentrations obtained in non-surgical eyes. Moxifloxacin concentrations were determined by HPLC. A total of six drops of moxifloxacin ophthalmic solution 0.5% was administered to each eye, as follows: 60 minutes before surgery three single drops (30 μl/drop) were applied separated by 5 minutes. The same regimen of three drops (each separated by 5 minutes) was applied at the end of surgery. The results of this study are shown in Table 5. This multi-drop regimen of moxifloxacin ophthalmic

TABLE 4

Animal Study: Fluoroquinolone Concentration in Aqueous Humor and Cornea Following a Single Topical Ocular Dose of 0.3% Solutions of Moxifloxacin and Ofloxacin to Dutch-belted Rabbits (F5)

	Time (hr)	Moxifloxacin Mean Conc (μg/mL) ± SD	Ofloxacin (μg/g) Mean Conc (μg/mL) ± SD
Aqueous humor	0.5	1.78 ± 0.39*	0.507 ± 0.489*
	1	0.993 ± 0.075	0.267 ± 0.134
	2	0.304 ± 0.059	0.229 ± 0.031
	4	0.0589 ± 0.0071	0.0933 ± 0.0389
	8	0.0353 ± 0.0232	BLQ
	12	0.0207 ± 0.0002	BLQ
	24	0.0189 ± 0.0099	BLQ
	48	0.0172 ± 0.0099	BLQ
Cornea	0.5	12.5 ± 3.8*	6.02 ± 2.27*
	1	5.89 ± 0.78	2.34 ± 0.99
	2	2.02 ± 0.13	2.41 ± 0.46
	4	0.65 ± 0.082	1.05 ± 0.39
	8	0.99 ± 0.288	0.493 ± 0.123
	12	0.437 ± 0.225	0.150 ± 0.042
	24	0.253 ± 0.119	0.270 ± 0.240
	48	0.247 ± 0.015	0.0967 ± 0.0839
Iris-ciliary body	0.5	6.26 ± 2.07	0.800 ± 0.360
	1	10.4 ± 5.6	0.653 ± 0.423
	2	8.54 ± 1.45	4.43 ± 1.99
	4	11.0 ± 1.7	5.42 ± 2.88*
	8	13.5 ± 4.7*	4.86 ± 1.57
	12	9.42 ± 3.76	2.92 ± 0.07
	24	10.7 ± 6.2	3.33 ± 1.01
	48	7.68 ± 2.14	2.78 ± 0.33
Plasma	0.5	0.0130 ± 0.0016*	0.0069 ± 0.0037
	1	0.0109 ± 0.0010	0.0080 ± 0.0010*
	2	0.0065 ± 0.0003	0.0038 ± 0.0003
	4	BLQ	BLQ
	8	BLQ	BLQ
	12	BLQ	BLQ
	24	BLQ	BLQ
	48	BLQ	BLQ

BLQ = Below Limit of Quantitation of 33 ng/g.
Note: Each mean value was average of three samples;
* designates the maximum concentration obtained for that antibiotic in that tissue.

solution 0.5% produced aqueous humor concentrations of 12.2 to 32.6 μg/ml that were well above the MICs of even resistant strains of the most common organisms implicated in post cataract surgery endophthalmitis (e.g., *Staphylococcus aureus* and coagulase negative *Staphylococcus*). Relatively high concentrations of moxifloxacin were detected in the aqueous humor of both groups at all time points. At all time points, the mean moxifloxacin concentrations are at least 200-fold greater than the MIC_{50} value for FQ-susceptible *Staphylococcus* and at least four-fold higher than MIC_{50} for FQ-resistant *Staphylococcus*. There were no statistically significant differences between surgical or nonsurgical eyes at any time-point, indicating that cataract surgery does not alter the penetration of moxifloxacin into aqueous or vitreous humor. However, the study demonstrated that topical moxifloxacin administered as three single drops, 60 minutes prior to surgery and immediately after surgery resulted in a mean aqueous concentration for moxifloxacin that was 200-fold higher than the median MIC for fluoroquinolone-susceptible isolates of *Staphylococcus aureus* or coagulase-negative *Staphylococcus*.

Ocular Penetration of 0.5% Moxifloxacin, 0.3% Ofloxacin, and 0.3% Gatifloxacin

This Alcon rabbit study (F6) measured the ocular pharmacokinetics of topical moxifloxacin 0.5% (VIGAMOX®), ofloxacin 0.3% (Ocuflox®), and gatifloxacin 0.3% (Zymar®) solutions following repeated

[F6] Roberston SM, Sanders M, Jasheway D, et al: Absorption and distribution of moxifloxacin, ofloxacin and gatifloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits. Invest Ophthalmol Vis Sci 45: E-Abstract 4906, 2004.

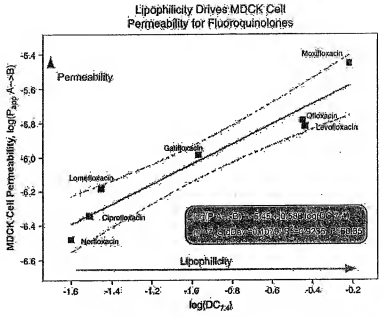

Fig. 1. Comparison of lipophilicity versus MDCK cell permeability (F2).

topical ocular doses of three times per day for 3 days to pigmented rabbits (F6). Male Dutch-belted rabbits received a thorough slit-lamp biomicroscopic examination prior to dosing. Only rabbits with no ocular defects were randomized into the three test groups. There were 30–33 rabbits per group. Each rabbit received bilateral 30-µl doses of VIGAMOX®, Ocuflox®, or Zymar® three times per day for 3 days. Doses were administered at 8 AM, 12 PM, and 4 PM on days 1 and 2; at 8 AM, 4 PM, and 12 AM on day 3; and at 8 AM on day 4. Prior to euthanasia, blood from each animal was collected and centrifuged for the plasma fraction. Three treated animals per time point from each group (three eye pairs or n = 6 eyes) were euthanized at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 144, and 288 hours, followed by collection of aqueous humor, cornea, iris-ciliary body, and vitreous humor (0.25 hour was excluded from the latter two tissues). Sample weights were recorded and all samples were frozen on dry ice prior to storage at −70°C.

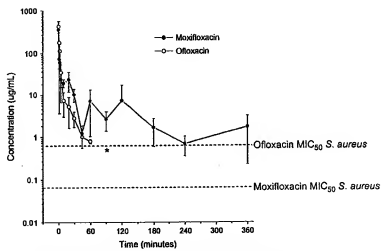

Fig. 2. Mean concentrations of fluoroquinolones in tear film of rabbits after a single topical dose of moxifloxacin or ofloxacin (F5).

TABLE 5

Animal Study: Effect of Cataract Surgery on Aqueous and Vitreous Humor Concentrations of Moxifloxacin in Rabbits[12]

	Time [hr]	Moxifloxacin in Aqueous Humor Mean Conc [μg/mL] ± SD	Moxifloxacin in Vitreous Humor Mean Conc [μg/mL] ± SD
Surgical group	30 min	13.9 ± 7.2	0.0668 ± 0.0592
	60 min	16.2 ± 3.12	0.0666 ± 0.0863
	120 mn	12.2 ± 5.1	0.400 ± 1.0833
Nonsurgical group	30 min	25.3 ± 16.8	0.0431 ± 0.1227
	60 min	32.6 ± 20.1	0.2000 ± 0.631
	120 mn	15.7 ± 15.0	0.0544 ± 0.0497

n = 18 eyes per arm; differences in moxifloxacin concentration in eyes between the surgical and nonsurgical group were compared using a Wilcoxon signed rank test. An alpha of 0.05 was used to determine statistical significance. There were no significant differences between surgical and nonsurgical groups (P > 0.68).

The analysis of drug concentrations in the tissues was performed by a reverse phase HPLC/fluorescent method. Ocular tissues were homogenized in water and moxifloxacin, ofloxacin, and gatifloxacin were isolated by extraction methods. Samples were analyzed by reverse phase liquid chromatography. Lower limits of quantitation were: aqueous humor (0.0025 μg/ml for all FQs), cornea (0.0174 μg/g for all FQs), iris-ciliary body (0.218 μg/g for moxifloxacin and ofloxacin; 0.0992 μg/g for gatifloxacin), vitreous humor (0.00041 μg/g for all FQs), and plasma (0.0005 μg/ml for all FQs). The results are presented in Table 6 and indicate that, as expected, all three fluoroquinolones penetrated into the eye. In all ocular tissues, moxifloxacin achieved the greatest maximal concentrations compared to ofloxacin and gatifloxacin. Gatifloxacin penetrated the least. Moxifloxacin achieved levels that were four- to five-fold more than gatifloxacin and two- to four-fold more than ofloxacin. All three compounds showed prolonged retention in the pigmented iris-ciliary body due to melanin binding, as expected for fluoroquinolones. These results show that moxifloxacin is well absorbed into the anterior ocular tissues and can be found in the vitreous humor after topical ocular application to a level greater than either ofloxacin or gatifloxacin (F6).

Penetration of Moxifloxacin and Gatifloxacin into Rabbit Aqueous Humor

The purpose of this published study by Levine[11] from the University of Arizona was to evaluate the aqueous penetration of the fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin. Twenty New Zealand white rabbits were divided into two groups and dosed with commercial topical preparations of moxifloxacin (VIGAMOX®) or gatifloxacin (Zymar®). Group 1 [keratitis protocol] received a dose every 15 minutes for 4 hours and the aqueous humor was sampled 10 minutes after last dose; group 2 (cataract prophylaxis protocol) was dosed four times a day for 10 days and the aqueous humor was sampled 1 hour after the last dose in 12 eyes and 24 hours after the last dose in 8 eyes. The concentrations of FQ in the aqueous samples were determined by HPLC. Results are shown in Table 7. The keratitis-dosing regimen produced mean moxifloxacin concentrations of 11.06 μg/ml that was significantly

TABLE 6

Animal Study Fluoroquinolone Concentrations [μg/mL or μg/g] in Five, Rabbit Tissues Following Multiple** Topical Ocular Dosing of VIGAMOX®, Zymar®, or Ocuflox® (F6)*

	Moxifloxacin		Ofloxacin		Gatifloxacin	
Aqueous humor	1.42 ± 0.60	30 min	0.405 ± 0.135	30 min	0.310 ± 0.075	60 min
Cornea	21.3 ± 8.6	15 min	8.01 ± 2.79	15 min	4.9 ± 0.70	15 min
Iris-ciliary body	35.0 ± 6.6	120 min	10.0 ± 3.0	120 min	12.6 ± 3.3	480 min
Vitreous humor	15.6 ± 18.4	60 min	3.27 ± 5.36	240 min	2.79 ± 3.64	60 min
Plasma	11.5 ± 2.2	30 min	9.59 ± 2.41	30 min	6.80 ± 1.74	30 min

* Maximum concentrations (Cmax) were measured in μg/mL for the aqueous and vitreous humors and plasma, and in μg/g for the corneas, and iris-ciliary bodies.

** Dosed three times a day for 3 days plus one drop on the 4th day [10 drops total]; three treated animals per time point from each group (i.e., three eye pairs or n = 6 eyes); samples were taken at 10–11 timepoints; there were a total of 30–33 rabbits dedicated to each treatment group.

TABLE 7

Animal Study: Mean Aqueous Humor Concentration of Moxifloxacin and Gatifloxacin Following Two Topical Ocular Dosing Regimens in Rabbits[11]

	Moxifloxacin				Gatifloxacin			
Dosing Protocol	n	Mean Concentration (μg/mL ± SD)	SEM	Range	n	Mean Concentration (μg/mL ± SD)	SEM	Range
Keratitis*	9	11.06 ± 3.55	1.18	7.66–18.87	8	7.57 ± 2.22	0.78	4.75–10.86
Prophylaxis**	6	1.75 ± 1.17	0.48	0.92–3.87	6	1.21 ± 0.72	0.29	0.44–2.14

SD = standard deviation; SEM = Standard error of the mean. Statistical analyses of the aqueous antibiotic concentrations was performed using a 2-tailed *t* test assuming equal variances.

* Moxifloxacin concentration significantly higher that gatifloxacin (P = 0.030) in the keratitis dosing regimen.

** No difference between concentrations of the two antibiotics (P = 0.359) in the prophyloxis dosing regimen.

higher (p = 0.03) than the mean concentration for gatifloxacin of 7.57 μg/ml. The 10-day cataract prophylaxis regimen yielded similar results of 1.75 μg/ml for moxifloxacin and 1.21 μg/ml for gatifloxacin.

HUMAN CLINICAL STUDIES

Ocular penetration of moxifloxacin in humans was assessed in patients undergoing ocular surgery by monitoring drug concentrations in samples of aqueous humor or vitreous humor. Conjunctival levels were also determined in a study in healthy volunteers.

Penetration into Human Aqueous Humor

Moxifloxacin and Gatifloxacin (Cataract)

In studies by Katz et al[8], (F7) (Krieger Eye Institute), cataract patients were randomized to one of two groups (30 patients per group) (F7). For regimen 1, patients received moxifloxacin ophthalmic solution 0.5% as one drop to the operative eye four times at 15-minute intervals on the day of surgery. For regimen 2, the patients received moxifloxacin ophthalmic solution 0.5% as one drop to the operative eye four times at 15-minute intervals on the day prior to surgery plus four additional doses at 15-minute intervals on the day of surgery (F7). One sample per subject was collected at one of the following timepoints: 0.25, 0.5, 1, 2, or 3 hours after the last dose. This unique study design allowed researchers to calculate concentrations over time, to estimate an area under the inhibitory curve (AUIC). Table 8 gives the comparative aqueous humor values for patients treated with VIGAMOX® or Zymar using this approach. The moxifloxacin aqueous humor Cmax was 2.3- to 3.1-fold higher than gatifloxacin and the $AUC_{0\text{-}5h}$ was significantly (P < 0.05) higher (>2-fold) than gatifloxacin for both regimens. These C_{max} values (1.55–1.61 μg/ml) were 25- to 30-fold above the median MIC for *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates from clinical cases of endophthalmitis. Moxifloxacin concentrations were 16–20 times above the MIC for these organisms even 3 hours after the last dose. There was no statistically significant difference between regimen 1 and regimen 2 (p > 0.05). The findings showed that the ocular penetration of moxifloxacin is at least two-fold greater than gatifloxacin with either regimen.

Kim et al[9] administered VIGAMOX® or Zymar® topically to the eyes of 50 cataract patients prior to their surgeries.[9] Dosage was one drop every 10 minutes for four doses beginning one hour before surgery. At the time of surgery, a 30-gauge cannula on a tuberculin syringe was used to acquire the specimens of aqueous humor. Antibiotic concentrations were determined by HPLC. The mean concentration of moxifloxacin in the aqueous humors was 1.80 ± 1.21 μg/mL while that for gatifloxacin was 0.48 ± 0.34 (Table 9). This three-fold difference in antibiotic concentration favoring moxifloxacin was statistically significant (P = 0.00003).

Moxifloxacin (Vitrectomy)

In another clinical study, Hariprasad et al[7] determined moxifloxacin concentrations in aqueous humors of 20 vitrectomy patients following topical administration of moxifloxacin ophthalmic solution 0.5% for 3 days at 2- or 6-hour intervals. Assays were performed using HPLC. The mean moxifloxacin concentrations in the aqueous humor are shown in Table 9. The mean concentrations were 2.28 and 0.88 μg/ml for the 2- and the 6-hour intervals, respectively for the aqueous humor. These values were significantly different (P = 0.01, t-test, two-tailed homoscedastic function). The moxifloxacin MIC_{90}s were far exceeded for a wide spectrum of pathogens

[F7] Katz HR, Lane S, Masket S, Sall K, Orr S, Foulkner R, Robertson SM, Dahlin DC: Human aqueous concentrations of moxifloxacin and gatifloxacin following two multiple-dose topical ocular dosing regimens with VIGAMOX® and Zymar. Invest Ophthalmol Vis Sci 46: E-Abstract 4907, 2005.

TABLE 8

Human Study: Moxifloxacin and Gatifloxacin Penetration into Aqueous Humor via Two Treatment Regimens[8] (F7)

	Moxifloxacin		Gatifloxacin	
Time	Regimen 1	Regimen 2	Regimen 1	Regimen 2
Cmax (μg/mL) ± SD	1.55 ± 0.86	1.61 ± 0.71	0.74 ± 0.66	0.91 ± 0.54
Tmax (min)	30	120	30	60
AUC_{0-3} (μg/mL) ± SE	2.99 ± 0.28	3.97 ± 0.44	1.79 ± 0.21	1.58 ± 0.25

SE = standard error; SD =standard deviation.

Regimen 1: Dosed 1 drop every 15 min for four doses prior to surgery [four drops total].

Regimen 2: Dosed 1 drop every 15 min for four doses prior to surgery plus four doses the day prior to surgery [eight drops total].

n = 30 for each of the four treatment groups; 120 aqueous humor samples were collected, 60 from patients randomized to each fluoroquinolone with 30 at each dosing regimen.

Moxifloxacin regimen 1 was significantly better than gatifloxacin regimen 1 ($P < 0.05$).

Moxifloxacin regimen 2 was significantly better than gatifloxacin regimen 2 ($P < 0.05$).

including *Staphylococcus epidermidis, S. aureus, Streptococcus pneumoniae, S. pyogenes, Propionibacterium acnes, Haemophilus influenzae,* and *Escherichia coli.*

Moxifloxacin and Gatifloxacin (Phacoemulsification)

McCulley, Aronowicz and co-investigators at the University of Texas Southwestern Medical Center determined the aqueous humor concentrations of moxifloxacin and gatifloxacin after five topical doses in humans undergoing planned phacoemulsification and IOL insertion (F8, F9, F10). Forty-six patients were dosed with one drop QID 1 day before surgery and one drop 1 hour prior to surgery. Aqueous humor samples were collected, frozen and subsequently analyzed by HPLC to determine the concentration of FQ. All participants were masked throughout the investigation. The results are given in Table 9. In their 46-patient study, Aronowicz et al (F8) showed that moxifloxacin accumulated at levels twice that of gatifloxacin in the aqueous humor: 1.86 ± 0.23 μg/ml for moxifloxacin vs. 0.94 ± 0.15 μg/ml for gatifloxacin; P = 0.001.

Moxifloxacin, Ciprofloxacin, and Ofloxacin (Cataract)

In this study, Solomon et al[14] compared moxifloxacin, ciprofloxacin, and ofloxacin in a double-masked cataract patient study involving 52 cataract patients receiving one of the three antibiotic products four times a day for 3 days prior to surgery and an additional four doses at 15-minute intervals in the hour prior to surgery (16 total doses). Aqueous humor (0.1 ml) was collected during the surgical procedure. The aspirate was immediately frozen at −70°C. Fluoroquinolone concentrations were determined by reverse phase HPLC. The mean aqueous humor drug concentrations are shown in Table 9. Both moxifloxacin ($P < 0.001$) and gatifloxacin ($P < 0.005$) penetrated into the aqueous humor significantly better than ciprofloxacin, while moxifloxacin also penetrated into the aqueous better than gatifloxacin ($P < 0.05$).

Summary of Aqueous Humor Penetration

A summary of the findings from 13 aqueous humor clinical studies is shown in Table 9.

Penetration into Human Vitreous Humor

In the Hariprasad et al study,[7] moxifloxacin concentrations were also determined in the vitreous humors of 20 vitrectomy patients following topical administration of moxifloxacin ophthalmic solution 0.5% for 3 days at 2- or 6-hour intervals.[7] The mean moxifloxacin concentrations were 0.11 and 0.06 μg/ml in the vitreous humor for the 2- and 6-hour regimens, respectively (Table 9). These values were not significantly different (P = 0.08, t-test, 2-tailed homscedastic function). These concentrations did not exceed the MIC_{90}s for these organisms but did exceed the MIC_{50} values. In contrast, Lott showed that

[8] Aronowicz JD, Shine W, McCulley JP: Aqueous humor concentrations of fourth-generation fluoroquinolones in humans. *Invest Ophthalmol Vis Sci* 46: E-Abstract 5051, 2005.

[9] McCulley JP, Surratt G, Shine W: 4th generation fluoroquinolone penetration into aqueous humor in humans. Invest Ophthalmol Vis Sci 45: E-Abstract 4927, 2004.

[10] McCulley JP, Shine WE: Comparative penetration of 2 fourth-generation fluoroquinolones into the aqueous humor of humans. Presented as Paper PA077 during the Oct 23-26, 2004 Cataract Free Paper Session of the American Academy of Ophthalmology Meeting in New Orleans, LA.

TABLE 9

Summary of Human Clinical Studies: Penetration of Moxifloxacin, Gatifloxacin, Ciprofloxacin, and Ofloxacin into Human Aqueous Humor

Reference	Patients	Topical Dosage	Fluoroquinolone Aqueous Humor Concentration μg/mL
Moxifloxacin 0.5% (VIGAMOX®)			
Hariprasad 2005[7]	9 Vitrectomy	1 drop every 2 hours for 3 days prior to surgery (43 drops)	2.28 ± 1.23
Hariprasad 2005[7]	10 Vitrectomy	1 drop every 6 hours for 3 days prior to surgery (22 drops)	0.88 ± 0.88
Kim 2005[9]	25 Cataract	1 drop every 10 minutes prior to surgery (4 drops)	1.80 ± 1.21
Solomon 2005[14]	14 Cataract	4 times/day for 3 days and 3 doses 1 hour before surgery (15 drops)	1.31 ±0.46
Aronowicz 2005(F8)	23 Phaco/IOL	4 times/day for day before surgery and 1 hour before surgery (5 drops)	1.86 ± 0.23
Katz 2005[8]	60 Cataract	Regimen 1: 4 drops pre-surgery (4 drops)	1.55 ± 0.86
Katz 2005[8]	60 Cataract	Regimen 2: 4 times/day for day before surgery and 4 drops pre-surgery (8 drops)	1.61 ± 0.71
Gatifloxacin 0.3 % (Zymar®)			
Chu 2004(F13)	25 cataract	4 times/day for 3 days and 1 drop every 15 minutes for 1.5 hours prior to surgery (18 drops)	1.10*
Kim 2005[9]	25 Cataract	1 drop every 10 minutes prior to surgery (4 drops)	0.48 ± 0.34
Price 2005[13]	10 Cataract	4x/day for 2 days before surgery and 1 drop every 10 minutes 1 hour before surgery (14 drops)	1.26 ± 0.55
Solomon 2005[14]	16 Cataract	4 times/day for 3 days and 3 drops 1 hour before surgery (15 drops)	0.63 ± 0.30
Aronowicz 2005(F8)	23 Phaco/IOL	4 times/day for 1 day before surgery and 1 hour before surgery (5 drops)	0.94 ± 0.15
Katz 2005[8]	60 Cataract	Regimen 1: 4 drops pre-surgery (4 drops)	0.74 ± 0.66
Katz 2005[8]	60 Cataract	Regimen 2: 4 times/day for day before surgery and 4 drops pre-surgery (8 drops)	0.91 ± 0.54
Ciprofloxacin 0.3% (Ciloxan®)			
Cekic 1999[2]	18 Vitrectomy	During 6 hours before surgery: 2 drops every 30 minutes for 1st 3 hours and every 60 minutes for next 3 hours (9 drops)	0.44 ± 0.07 SEM
Solomon 2005[14]	22 Cataract	4 times/day for 3 days and 3 doses 1 hour before surgery (15 drops)	0.15 ± 0.11
Donnenfeld 1994[4]	12 Cataract	2 drops 90 minutes preop and 2 drops 30 minutes postop (4 drops)	0.072*
Perry 2004(F14)	14 Cataract	4 times/day for 3 days prior to surgery plus every 15 minutes 1 hour prior to surgery (16 drops)	0.11 ± 0.04
Ofloxacin 0.3% (Ocuflox®)			
Cekic 1998[3]	14 Vitrectomy	During 6 hours before surgery: 2 drops every 30 minutes for 1st 3 hours and every 60 minutes for next 3 hours (9 drops)	1.44 ± 0.24 SEM
Donnenfeld 1994[4]	12 Cataract	2 drops 90 minutes preop and 2 drops 30 minutes postop (4 drops)	0.338
Perry 2004(F14)	15 Cataract	4 times/day for 3 days prior to surgery plus every 15 min 1 hour prior to surgery (16 drops)	0.31 ± 0.62
Levofloxacin 0.5% (Quixin®)			
Yamada 2002[16]	20 Cataract	3 drops every 15 minutes 90 minutes presurgery (18 drops)	1.00 ± 0.48
Perry 2004(F14)	14 Cataract	4 times/day for 3 days prior to surgery plus every 15 minutes 1 hour prior to surgery (16 drops)	0.49 ± 0.79

SEM = standard error of mean.
* SD, SEM, or SE not reported.

the vitreous concentration of orally administered moxifloxacin (two 400-mg doses during 18 hours prior to surgery) produced mean levels in the vitreous of 0.86 μg/ml in nine patients (F11).

Penetration into Human Conjunctivae

A recent, unique pharmacokinetic study by Wagner and associates[13] (F3). Healthy volunteers were administered a single drop of either moxifloxacin 0.5%

TABLE 10

Human Study: Concentration of 5 Fluoroquinolones in Human Conjunctivae Following Topical Dosing

Reference	Mean FQ Conc in Human Conjunctivae [μg/g ± SD]				
	Moxifloxacin	Gatifloxacin	Ciprofloxacin	Levofloxacin	Ofloxacin
Wagner 2005[15] (F16)	18.00 ± 16.4	2.54 ± 2.99	2.65 ± 2.01	2.34*	1.26 ± 0.88
Number of patients	15	14	12	12	13

* Standard deviation, standard error of the mean, or standard error not reported.

TABLE 11

Summary of Human Clinical Studies: Topical Penetration of Moxifloxacin, Ciprofloxacin and Ofloxacin into Human Vitreous Humor

Reference	Patients	Topical Dosage	Fluoroquinolone Vitreous Humor Concentration μg/mL*
Moxifloxacin 0.5% (VIGAMOX®) Hariprasad 2005[7]	10 vitrectomy	1 drop every 2 hours for 3 days prior to surgery (43 drops)	0.11 ± 0.05
Hariprasad 2005[7]	9 vitrectoniy	1 drop every 6 hours for 3 days prior to surgery (22 drops)	0.06 ± 0.06
Ciprofloxacin 0.3% (Ciloxan®) Cekic 1999[2]	18 vitrectomy	During 6 hours before surgery: 2 drops every 30 minutes for 1st 3 hours and every 60 minutes for next 3 hours (9 drops)	0.22 ± 0.04 (SE)
Ofloxacin 0.3% (Ocuflox®) Cekic 1998[5]	14 vitrectomy	During 6 hours before surgery: 2 drops every 30 minutes for 1st 3 hours and every 60 minutes for next 3 hours (9 drops)	0.37 ± 0.05 (SE)

SE = standard error.

* Mean FQ Conc in μg/mL ± SD unless otherwise indicated.

(VIGAMOX®), ciprofloxacin 0.3% (Ciloxan® [Alcon Laboratories, Inc., Fort Worth, TX]), gatifloxacin 0.5% (Zymar®), ofloxacin 0.3% (Oculoflox®), or levofloxacin 0.5% (Quixin®) topically. Conjunctival biopsies were taken from the dosed eye (one each from temporal and nasal regions of the inferior cul de sac) 20 minutes post-dose. The biopsy and analytical methods used in this study represent a novel, safe and accurate technique for obtaining conjunctival tissue antibiotic concentrations. The specimens were analyzed by a dual analyte reverse phase HPLC method. Subjects were also followed for 1 week. The mean achievable tissue concentrations obtained in the conjunctivae are given in Table 10. The mean concentration (Cmax) of moxifloxacin in the conjunctiva 20 minutes post dose was approximately 18 μg/g as compared to 2.5 μg/g with gatifloxacin. This concentration for moxifloxacin was six- to 14-fold higher than that achieved for either ciprofloxacin (6.8-fold), gatifloxacin (7.1-fold), ofloxacin (14.6-fold), or levofloxacin (7.7-fold). The conjunctival levels of moxifloxacin were statistically significantly higher than those of the other four FQs ($P < 0.001$). There was no statistically significant difference between the conjunctival concentrations of the other four FQs when moxifloxacin was excluded from the analysis ($P = 0.3549$). The investigators suggest that the enhanced penetration may result from moxifloxacin's high biphasic solubility (both lipophilic and aqueous solubility).

Discussion/Conclusions

Data across multiple *in vitro, ex vivo,* animal and human clinical studies consistently demonstrate a trend of superior ocular penetration of moxifloxacin compared to other topical fluoroquinolones. This publication includes the major findings and conclusions of previously published or abstracted work as well as original data collected by Alcon.

In vitro and *ex vivo* studies indicate that this difference is due to the molecular structure of moxifloxacin and specifically its high lipophilicity combined with its high aqueous solubility. Moxifloxacin is unusual among fourth-generation fluoroquinolones in having a bicyclic amine side chain at the C7

[F1] Lott MN, Fuller JJ, Robertson SM, Curtis MA, Dahlix DC, Singh H, Marcus DM: Vitreous penetration of orally-administered moxifloxacin in humans. Invest Ophthalmol Vis Sci 46: E-Abstract 4896, 2005.

position, conferring hydrophilicity along with lipophilicity. In *in vitro/ex vivo* models, moxifloxacin penetrated corneal tissues 3.6-fold better than gatifloxacin and two-fold faster. VIGAMOX® maintained corneal integrity better than Zymar®. Moxifloxacin was the most lipophilic fluoroquinolone of the seven fluoroquinolones tested and showed the greatest aqueous solubility. The presence of the bulky bicyclo amine substituent inhibits the bacterial active efflux pump (F12). As a result, moxifloxacin has sufficient lipophilicity to readily cross the corneal epithelium while simultaneously it has high aqueous solubility and potency at physiological pH. For example, moxifloxacin has an aqueous solubility at pH 7 of up to at least 3.0%. In contrast, the maximum solubility of ciprofloxacin at this pH is about 0.01%. The high solubility of moxifloxacin results in higher tear film concentrations providing a driving force (concentration gradient) for corneal uptake.

Various *in vivo* animal and human clinical studies support the fact that moxifloxacin penetrates ocular tissues better than other fluoroquinolones when instilled topically. In the single-dose animal studies, the instillation of a single drop topically of a 0.3% solution of moxifloxacin achieved maximum levels in the rabbit cornea, aqueous humor and iris-ciliary bodies of 12.5, 1.8, and 13.5 μg/ml or g, respectively. Concentrations of moxifloxacin were typically two-fold higher than the corresponding values for ofloxacin and remained two-fold higher throughout the study. Moxifloxacin concentration in the cornea was 0.25 μg/g at 48 hours, or about four-fold above the MIC for methicillin-susceptible *Staphylococcus aureus*. In contrast, ofloxacin corneal concentrations were below its MIC threshold by 8 hours. The mean moxifloxacin tear film concentration at the initial 10-minute time-point was 366 μg/ml and remained at or above 1 μg/ml for up to at least 6 hours post-dose. Plasma concentrations of both drugs were low (0.01 μg/ml or less) and declined rapidly. Moxifloxacin exhibited a better penetration profile than ofloxacin which penetrates better than ciprofloxacin. Maximal moxifloxacin levels in ocular tissues were typically two-fold higher than ofloxacin levels and generally remained higher than those of ofloxacin over time. In the cornea, moxifloxacin levels were about four-fold higher than the MIC_{90}s for MRSA strains even at 48 hours post-dose, whereas ofloxacin levels fell below its MIC_{90} by 8 hours. After topical dosing, tear film concentrations of moxifloxacin remained higher (seven- to 10-fold) than ofloxacin over time. Ofloxacin levels at 90 minutes were below the quantitation limits, but moxifloxacin remained at or above 1 μg/ml up to the last time point at 6 hours. Systemic exposure was low following topical ocular administration. Based on plasma levels determined in toxicology and clinical studies at well tolerated doses, 0.3% moxifloxacin solution has a wide margin of safety.

In multidose animal studies by Robertson, moxifloxacin exhibited a better penetration profile than gatifloxacin or ofloxacin (F1). Maximal moxifloxacin levels in the ocular tissues were typically three- to six-fold higher than ofloxacin or gatifloxacin. Upon topical instillation, moxifloxacin achieved levels of 0.2 to 0.4 μg/ml in the vitreous humor in animals or 0.06 to 0.11μg/ml in humans. These levels were considerably lower than those obtained in the aqueous in these studies, but exceeded MIC_{50} for moxifloxacin against *Staphylococcus epidermidis, S. aureus, Streptococcus pneumoniae, Haemophilus influenzae* and other gram-negative bacteria. Although MICs are related to the efficacy in the treatment or prevention of infection, they are not always predictive. For example, even though moxifloxacin has a higher MIC against *Pseudomonas aeruginosa, in vivo* infections studies at Wilmer Eye Institute have shown that topical moxifloxacin 0.5% was as effective as ciprofloxacin 0.3% in treating infections with this particularly troublesome organism.[1]

These topical penetration studies in animals fairly well predicted what happens in humans. The rabbit data clearly give an edge to moxifloxacin as the fluoroquinolone that penetrates the best into the aqueous after topical treatment. This is also seen in the human clinical trials summarized in Tables 10 and 11. Although there are some low accumulations of fluoroquinolones in the vitreous humor via the topical route, the concentrations are at or near the MICs of susceptible strains of pathogens, but the levels are not high enough for therapeutic considerations (Table 11).[2,5,7] Nevertheless, there is extensive penetration of moxifloxacin into the human conjunctiva after topical instillation (Table 10)[15] (F3). This is not surprising, since Gipson demonstrated the similarity of the cell-to-cell and cell-to-substrate junctions between cornea and conjunctiva.[6]

The concentration of fluoroquinolone present in commercial products certainly influences relative potency of products. Upon topical administration of moxifloxacin ophthalmic solution 0.5%, moxifloxacin penetrates ocular tissues more extensively than

[12] Avelox® Package Insert, Bayer Pharmaceuticals Corporation, 2004.

[13] Chu Y: Penetration of gatifloxacin ophthalmic solution 0.3% into aqueous humor of patients undergoing cataract surgery. Invest Ophthalmol Vis Sci 45: E-Abstract 4007, 2004.

[14] Perry HD, Donnenfeld E, Bloom A, Snyder R, Levine J, Mychajlyszyn J, Greenman H, Solomon R: Aqueous humor concentrations of topically applied fluoroquinolones. Invest Ophthalmol Vis Sci 45: E-Abstract 4932, 2004.

other topical fluoroquinolones, giving substantial and prolonged aqueous humor, corneal, iris-ciliary body, and tear concentrations well above the MICs for common ocular pathogens.[10] The higher concentration of moxifloxacin in the commercial preparation (i.e., VIGAMOX® with moxifloxacin at 0.5%) compared to 0.3% gatifloxacin in Zymar®, 0.3% ciprofloxacin in Ciloxan®, or 0.3% ofloxacin in Ocuflox also enhances its penetration.

Method of Literature Search

We performed an international literature search for this article based on MEDLINE database searches from 1990 to 2005, using varying combinations of the search terms: *moxifloxacin, fluoroquinolones, ocular penetration, aqueous humor.* All relevant journal articles and/or abstracts were selected for review. English abstracts were used for non-English papers. Recent papers presented at ARVO were also included for completeness.

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ELSEVIER

Safety of Moxifloxacin as Shown in Animal and *In Vitro* Studies

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Abstract. Topical treatment of ocular bacterial infection is practiced widely, and the choice of the antibacterial agent depends on the nature of the infection, including the susceptibility of the organism, the tissue affected, and the safety profile of the agent. Moxifloxacin is a fourth-generation fluoroquinolone approved for ophthalmic use as moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon, Fort Worth, TX). Moxifloxacin ophthalmic solution 0.5% is self-preserved at a near-neutral pH of 6.8. In treating ocular infection, the three important aspects of therapeutic control are potency, penetration of the drug to the target site, and safety of the drug and the drug product. Moxifloxacin ophthalmic solution 0.5% provides antibacterial potency and high penetration of target ocular tissues. The ocular and systemic safety profile of moxifloxacin compares favorably with those of other fluoroquinolone antimicrobial agents, with a low risk of recognized quinolone-related toxicity. *In vitro* studies of fluoroquinolones with human or rabbit corneal epithelial cells or keratocytes suggest that moxifloxacin is similar in cytotoxicity potential to other drugs of this family. Specialized *in vivo* corneal wound-healing studies draw little distinction between moxifloxacin-treated eyes and those treated with other fluoroquinolones. Repeated-dose topical ocular studies in rabbits and monkeys, with high concentrations (up to 3%) of moxifloxacin and at treatment durations and regimens well in excess of label-prescribed use, demonstrated a high safety margin for ocular and extraocular tissues. Cornea, the tissue with highest exposure, was found to be unaffected by these high exposures, with slit-lamp biomicroscopy, corneal thickness measurement, intraocular pressure, and specular microscopy of the corneal endothelium (monkeys only), and histologic evaluation showing no effects, as compared with controls. Moxifloxacin ophthalmic solution 0.5% affords superior efficacy and ocular tissue penetration, with a favorable safety profile. (**Surv Ophthalmol 50:S46–S54, 2005.**

Key words. fluoroquinolone • moxifloxacin • nonclinical safety • safety pharmacology • toxicity • VIGAMOX®

Drug safety is assessed through a rigorous nonclinical testing program in drug development. It is estimated that five new medicinal entities advance to testing in humans out of 5,000 candidate drugs, and only one of these five may ultimately gain regulatory approval. Nonclinical assessment includes efficacy and safety pharmacology, pharmacokinetics/metabolism, and toxicology studies. Fluoroquinolones are generally considered safe and well tolerated, as compared with other commonly prescribed antimicrobials.[6]

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TABLE 1

Product Synopsis: Ophthalmic Fluoroquinolones[*]

	Ciloxan®	Ocuflox®	Quixin®	VIGAMOX®	Zymar®
Drug	Ciprofloxacin 0.3%	Ofloxacin 0.3%	Levofloxacin 0.5%	Moxifloxacin 0.5%	Gatifloxacin 0.3%
Benzalkonium chloride	0.006%	0.005%	0.005%	–	0.005%
Edetate disodium	0.05%	–	–	–	+
Sodium chloride	+	+	+	+	+
Sodium acetate	+	–	–	–	–
Acetic acid	+	–	–	–	–
Boric acid	–	–	–	+	–
Mannitol	4.6%	–	–	–	–
pH	4.5	6.4	6.5	6.8	6
Osmolality	300	300	300	290	260–330
Dose regimen initial	Days 1 and 2: 1–2 drops every 2 hours	Days 1 and 2: 1–2 drops every 2–4 hours	Days 1 and 2: 1–2 drops every 2 hours	1 drop t.i.d., 7 days (38-μL drop)	Days 1 and 2: 1 drop every 2 hours
Follow-up	Days 3–7: 1–2 drops every 4 hours	Days 3–7: 1–2 drops 4 times a day	Days 3–7: 1–2 drops 4 times a day		Days 3–7: 1–2 drops 4 times a day
Plasma C_{max} (ng/mL)	2.5	1.9	2.25	2.7	<5

+ = present in the product, concentration not specified; – = not present in the product; C_{max} = peak concentration achieved in the tissue.

[*] PDR Electronic Library Online: Thomson MICROMEDEX, http://www.thomsonhc.com/pdrel/librarian/ND_PR/Pdr, 2004.

Structural refinements have improved the safety and efficacy profiles of the new fourth-generation fluoroquinolones (i.e., moxifloxacin, gatifloxacin, and trovafloxacin). The approval of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon, Fort Worth, TX) and gatifloxacin ophthalmic solution 0.3% (Zymar®, Allergan, Irvine, CA) by the US Food and Drug Administration in 2003 added two powerful fourth-generation fluoroquinolones to the ophthalmologist's armamentarium. These new drugs offer high potency against ocular pathogens, with spectrum advantages over the earlier-generation ophthalmic fluoroquinolones ciprofloxacin ophthalmic solution 0.3% (Ciloxan®, Alcon), ofloxacin ophthalmic solution 0.3% (Ocuflox®, Allergan), and levofloxacin ophthalmic solution 0.5% (Quixin, Vistakon, Jacksonville, FL). Moxifloxacin ophthalmic solution 0.5% is an iso-osmotic solution containing 0.5% moxifloxacin, boric acid, and sodium chloride, with an approximate pH of 6.8. Moxifloxacin ophthalmic solution 0.5% is self-preserved, with no designated preservative added (F1). Gatifloxacin ophthalmic solution 0.3% contains gatifloxacin at a concentration of 0.3%, with benzalkonium chloride 0.005% as a preservative, ethylenediaminetetra-acetic acid, and sodium chloride. The solution is approximately pH 6 (no pH buffer added) and iso-osmotic. A synopsis of product characteristics is presented in Table 1. Moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3% combine the advantages of high local concentrations of antibiotic at the site of action with low systemic exposure levels after therapeutic use. Plasma drug levels associated with topical dosing are about 1,000-fold below those encountered with systemic use of fluoroquinolones. Therefore, these factors suggest that the topical application of these antibiotics do not contribute to systemic toxicity or the development of antimicrobial resistance.

Nonclinical Studies

Nonclinical studies conducted to assess the safety of moxifloxacin summarized here include safety pharmacology studies on major physiological systems and drug interaction potential, and toxicology studies to identify target organs and estimate safety margins.

SAFETY PHARMACOLOGY STUDIES

Safety pharmacology studies profiled effects of moxifloxacin on major organ systems, with particular attention to those known to be most affected—for example, the central nervous system, gastrointestinal tract, and cardiovascular system. Selected safety pharmacology study results are summarized in Table 2. The safety margins determined in these studies ranged from 400- to 7,000-fold, providing strong support that ophthalmic use of moxifloxacin is unlikely to result in significant adverse effects in humans.

[F1] Schlech BA, Sutton S, Rosenthal RA, et al: Antimicrobial preservative effectiveness of VIGAMOX®™ (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4913, 2004.

TABLE 2

Safety Pharmacology Studies for Moxifloxacin

System/Study Type	Species/Route	No Effect Dose*	Safety Margin†
Central nervous system			
Analgesia	Mouse/oral	100 mg/kg	>4,300 ×
Sensorimotor	Mouse/oral	30 mg/kg	>1,300 ×
Convulsant	Mouse/oral	30 mg/kg	>1,300 ×
Psychomotor	Rat/oral	30 mg/kg	>1,300 ×
Gastrointestinal			
Gastric acid secretion	Rat/intraduodenal	100 mg/kg	>4,300 ×
Indomethacin-induced erosin	Rat/oral	100 mg/kg	>4,300 ×
Acetylcholine, serotonin, histamine, $BaCl_2$-induced spasm	Guinea pig ileum/*in vitro*	10 μg/mL	>3,000 ×
Cardiovascular			
HERG channel (repolarization)	CHO cells/ *in vitro*	30 μM	>4,000 ×
Papillary muscle	Guinea pig/ *in vitro*	50 μM	>7,000 ×
Isolated cardiac myocyte assay	Guinea pig/ *in vitro*	50 μM	>7,000 ×
Torsades-de-pointes model	Rabbit/IV	120 mg/kg in 1 hour	>5,000 ×
ECG (QT/QTc interval)	Dog/IV	10 mg/kg	>430 ×
	Monkey/intraduodenal	100 mg/kg	>4,300 ×
Renal			
Urine volume and electrolyte excretion	Rat/oral	100 mg/kg	>4,300 ×
	Rat/IV	10 mg/kg (small change in K^+ at 30 mg/kg)	>430 ×
Respiratory			
Pulmonary resistance, lung compliance, and respiration rate	Guinea pig/oral	100 mg/kg	>4,300 ×
HR, BP, respiration rate, pulmonary function	Guinea pig/IV	10 mg/kg	>430 ×
Other			
Blood glucose levels	Rat/IV	30 mg/kg	>1,300 ×

BP = blood pressure; CHO = Chinese hamster ovary; ECG = electrocardiogram; HERG = human ether-a-go-go–related gene; HR = heart rate; IV = intravenous.

* That elicited no statistically or biologically significant pharmacologic response.

† Ratio of no effect dose (mg/kg) to clinical dose (0.023 mg/kg), (based on 100% absorption of 1 drop 0.5% moxifloxacin solution, both eyes, three times a day, to a 50 kg patient) for the *in vivo* studies, or mean clinical C_{max} (2.7 ng/mL) for *in vitro* studies using exposure by concentration (μM or μg/mL).

Moxifloxacin produced no overt central nervous system effects in rats or mice at a 30-mg/kg dose in a battery of central nervous system screening studies for analgesia, convulsant, sensorimotor, and psychomotor activity. Sedation was observed in rodents at the highest oral dose tested (100 mg/kg), with no effect observed at 30 mg/kg.

Fluoroquinolone antibiotics are recognized to produce prolongation of the QTc interval of the electrocardiogram, and *in vitro* and *in vivo* studies were conducted to assess this potential for moxifloxacin. These studies were performed by Alcon and Bayer in support of the US New Drug Application of VIGAMOX® and included guinea pig papillary muscle action potential duration, canine Purkinje fiber action potentials, patch clamp studies, human ether-a-go-go–related gene (HERG) channel studies, a special rabbit model of torsades de pointes, and electrocardiogram assessments in dogs and monkeys. Intravenously infused moxifloxacin (10 mg/kg) produced no electrocardiogram changes in dogs, and no significant human ether-a-go-go–related gene channel inhibition was observed with high concentrations of moxifloxacin.[11] Safety pharmacology studies thus demonstrated high therapeutic indices for physiological systems of concern with fluoroquinolone use, emphasizing the benefit of local antibacterial efficacy with minimal systemic risk.

TOXICOLOGY STUDIES

Toxicology studies were conducted to establish the pharmacotoxicological profile of moxifloxacin, to assure safe use in clinical trials and to support drug safety for worldwide marketing applications. Systemic toxicity studies conducted by Bayer established the safety profile for moxifloxacin in support of oral and intravenous drug products (Avelox®, Bayer). Additional studies were conducted by Alcon to assess safety with topical ophthalmic use. Further investigations

relating to special-use situations have been reported in published literature. The systemic and general toxicology profile of moxifloxacin has been described.[19] Principal target organs were identified as the liver, heart, central nervous system, and bone marrow. "No effect" doses were well in excess of therapeutic doses for systemic use and generally ranged from 30 to 100 mg/kg/day. Toxicity specifically associated with fluoroquinolones (e.g., phototoxicity, arthropathy, electrocardiogram changes) was observed only at relatively high doses (>30 mg/kg). No effects were observed on fertility, reproduction, or embryo-fetal development at doses of 30 mg/kg or below. Genotoxic effects were seen in some assays, but these effects are common to all fluoroquinolones and related to the antimicrobial mechanism of action of the drug. An accelerated carcinogenicity bioassay suggested no tumorigenic activity.

OCULAR STUDIES

In Vivo Studies

Drug exposure by topical ophthalmic use is low compared with systemic doses. The plasma peak concentration achieved in the tissue for systemic therapy is 4–5 μg/mL, whereas the average highest plasma concentrations in subjects receiving topical moxifloxacin ophthalmic solution 0.5% is reported to be 2.7 ng/mL—a difference of more than 1,000-fold.

An eyedrop delivers an initial concentration of drug equivalent to that of the product. It is estimated that most of an administered eyedrop is lost to drainage in the first 15 to 30 seconds after instillation.[17] The tear turnover rate is approximately 16% per minute, so that nearly all of the drug is expected to disappear within 10 minutes after dosing. Maurice and Mishima estimated that the instilled drug is diluted in the tear fluid, and after drainage of excess fluid, the drug concentration averages about one third of the original drop.[14] Robertson et al showed that the concentration of moxifloxacin in tears following a single dose of moxifloxacin ophthalmic solution 0.5% in rabbits was approximately 400 μg/mL initially, but dropped to 1–10 μg/mL by about 30 minutes post-instillation (**F2**). Thus, exposure of ocular tissue, including corneal epithelium, to the drug at the product concentration will be very short-lived.

Nonclinical studies to assess the safety of moxifloxacin ophthalmic solution were conducted in rabbits and monkeys by the clinical route of administration. In these studies, standard animal models were used with high exposure to the drug (by increased drug concentration, dose regimen, and/or treatment duration) and incorporated thorough ophthalmologic and systemic assessments. These measures help establish safety margins for both systemic exposure and local effects on the eye and ocular adnexa.

Two studies employed different rabbit varieties, the albino New Zealand White and a pigmented NZ White × NZ Red cross (**F3**). A pigmented variety was desired because fluoroquinolones possess high binding affinity to melanin. In both studies, animals were dosed with two drops, to the right eye, four times a day, for approximately 1 month. Treatments were 0% (vehicle), 0.5%, 1.0%, and 3.0% moxifloxacin solutions. Ocular evaluations, including slit-lamp biomicroscopy, corneal thickness measurements, indirect ophthalmoscopy, and microscopic evaluation, revealed no significant effects. Systemic parameters, such as body weight, general health, clinical laboratory tests, and histopathology, also showed no significant treatment-related effects.

A subchronic ophthalmic safety study in cynomolgus monkeys employed a dosing regimen of two drops to the right eye, six times a day for 16 days, followed by t.i.d. dosing for the remainder of the 3-month study (**F4**). Treatments were 0% (vehicle), 0.5%, 1.0%, and 3.0% moxifloxacin solutions. Ocular and systemic evaluations included those described for the rabbit studies, as well as intraocular pressure and specular microscopy of the corneal endothelium. All ophthalmic parameters were comparable in the untreated eyes, vehicle controls, and moxifloxacin ophthalmic solution–treated eyes. Corneal thickness, a sensitive indicator of corneal health, was not affected by administration of moxifloxacin ophthalmic solution, even at the high concentrations and extreme regimen employed. Mean corneal endothelial cell density and cell area were comparable in vehicle control and moxifloxacin-treated eyes, further establishing corneal safety. No significant findings were observed for any systemic parameters.

The effects of topical administration of moxifloxacin and gatifloxacin ophthalmic solutions have been investigated with alternative treatment regimens. These studies employed normal healthy corneas. Herrygers et al reported that New Zealand White rabbits dosed with moxifloxacin ophthalmic solution 0.5% under either a keratitis regimen (one drop

[F2] Robertson SM, Sanders M, Jasheway D, et al: Penetration and distribution of moxifloxacin and ofloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits (abstract). Invest Ophthalmol Vis Sci 44(Suppl):1454, 2003.

[F3] McGee DH, Heaton J, Hackett R, et al: Toxicity of moxifloxacin ophthalmic solution 0.5% in the rabbit (abstract). Invest Ophthalmol Vis Sci 44(Suppl):4458, 2003.

[F4] Bergamini MV, Heaton J, McGee D, et al: A three-month topical ocular toxicity study of moxifloxacin ophthalmic solutions in cynomolgus monkeys (abstract). Invest Ophthalmol Vis Sci 44(Suppl):4457, 2003.

every 5 minutes for 15 minutes, followed by one drop every 15 minutes for 4 hours) or a post–cataract surgery prophylaxis regimen (four times daily for 10 days) showed no significant corneal epithelial damage by scanning electron microscopic evaluation by either regimen.[9] Similar results were seen with gatifloxacin ophthalmic solution 0.3% and vehicle in this study, and it was concluded that both products were well tolerated by the ocular surface and considered safe and nontoxic. The effects of ophthalmic solutions of moxifloxacin, ciprofloxacin, levofloxacin, and ofloxacin on corneal epithelium and stroma were assessed by confocal microscopy in rabbits.[12] All products except moxifloxacin ophthalmic solution 0.5% contain 0.005% or 0.006% benzalkonium chloride. Tears Naturale Free (Alcon) was used as a control. After 6 days of treatment, corneal epithelial thickness was decreased from baseline for all drug-treated groups except moxifloxacin ophthalmic solution 0.5% and Tears Naturale Free, whereas corneal stromal thickness was similar to baseline.

Corneal Wound Healing Studies

Some studies have suggested effects of fluoroquinolones on corneal wound healing. Heavy dosing of de-epithelialized rabbit corneas with ofloxacin solution inhibited epithelial cell regrowth and resulted in keratocyte loss, as compared with both untreated and intact corneas.[15] Levofloxacin delayed re-epithelialization and resulted in some keratocyte loss, stromal swelling, and disorganization when dosed to de-epithelialized corneas of rabbits at high (3% and 6%) concentrations.[2] Delayed re-epithelialization, increased corneal thickness, and haze were observed in monkeys with the same treatment. These results suggest that ofloxacin and levofloxacin, at high doses, may result in some slight effects on corneal recovery.

Nonclinical studies have also been conducted to assess the safety of fourth-generation fluoroquinolones in the wounded cornea. Some reports have suggested that moxifloxacin ophthalmic solution 0.5% affects corneal wound healing. The healing of linear incisions and anterior keratectomy wounds were reported to be slightly slower in rabbit corneas treated with 0.5% moxifloxacin solution, as compared with 0.3% gatifloxacin solution (**F7, F6**).

However, an increasing number of nonclinical studies have shown no differences in corneal wound healing between moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3%. McCartney et al reported no differences were observed in slit-lamp biomicroscopy scores, histology, or electron microscopy between rabbit corneas treated with moxifloxacin ophthalmic solution 0.5% (one drop t.i.d. for 7 days) and those treated with gatifloxacin ophthalmic solution 0.3% (one drop four times a day for 7 days) after penetrating linear incisions (**F7**).

Sorour et al compared corneal healing with treatment of moxifloxacin, gatifloxacin, or levofloxacin (one drop four times a day; concentrations not stated in abstract) after photorefractive keratectomy in chicken eyes (**F8**). Chickens were chosen as an experimental model because the chicken eye possesses a prominent Bowman's layer and its cornea is histologically similar to the human cornea (F9). Wound sizes over time were comparable in the balanced salt solution control (56.69 hours), moxifloxacin (56.73 hours), and gatifloxacin (56.40 hours) and greater than with levofloxacin (65.64 hours). The overall healing rate over time did not statistically differ between treatment groups. Wound size and percent healing were statistically significantly less for levofloxacin compared with other groups at 60 and 66 hours postoperatively, and the fourth-generation fluoroquinolones appeared slightly less toxic on the epithelium than levofloxacin.

The healing rate was slightly higher for anterior keratectomy wounds in New Zealand White rabbits treated with one drop four times a day for 4 days with moxifloxacin ophthalmic solution 0.5% (87±8%) relative to gatifloxacin ophthalmic solution 0.3% (77±10%), with no significant differences in collagen IV expression (**F10**). Similar results were obtained in the corneas of pigmented rabbits, where

[F5] Schmidt L, Beuerman R: Comparison of gatifloxacin and moxifloxacin in healing of a linear incision in the rabbit cornea (abstract). Invest Ophthalmol Vis Sci 45(Suppl):1427, 2004.

[F6] Gao J, Siemasko K, Vu C, et al: Effect of 4th generation fluoroquinolone on rabbit corneal wound healing (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4889, 2004.

[F7] McCartney MD, Rice RL, Hackett RB, et al: Comparison of wound healing in pigmented rabbits receiving moxifloxacin 0.5% or gatifloxacin 0.3% ophthalmic solutions following penetrating corneal incision. Cornea. Submitted for publication.

[F8] Sorour H, Yee S, Chuang A, et al: Epithelial healing of the topical levofloxacin, gatifloxacin and moxifloxacin after photorefractive keratectomy in chickens and relative toxicity of topical levofloxacin, gatifloxacin, moxifloxacin and on ofloxacin on human corneal epithelial cell culture. Cornea. Submitted for publication.

[F9] Fowler WC: An animal model for LASIK flap research: the white leghorn chicken (abstract). Invest Ophthalmol Vis Sci 41(Suppl):S459, 2000.

[F10] Williams KK, Munger RJ, Shepard AR, et al: Corneal wound healing in New Zealand White rabbits following anterior keratectomy and treatment with moxifloxacin 0.5% ophthalmic solution or gatifloxacin 0.3% ophthalmic solution. Cornea. Submitted for publication.

wound healing percentages were 90±8% and 81±14% for moxifloxacin ophthalmic solution 0.5%–treated and gatifloxacin ophthalmic solution 0.3%–treated eyes, respectively (**F11**).

Most *in vivo* studies in animal models suggest that there are no significant adverse effects of the fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin. Indeed, the fourth-generation fluoroquinolones may offer advantages over some older products such as ofloxacin and levofloxacin.

In Vitro Studies

Cutarelli et al compared antimicrobial activity and *in vitro* corneal epithelial toxicity of five fluoroquinolones, two aminoglycosides, and cefazolin, and established that fluoroquinolones were generally more potent against ocular pathogens and less toxic to the corneal epithelial cells.[3] Reports from *in vitro* studies have suggested variable results in relative cytotoxicity when comparing multiple fluoroquinolones. Recently, Yee et al compared the effects of moxifloxacin and gatifloxacin with those of levofloxacin and ofloxacin ophthalmic solutions on human corneal epithelial cells *in vitro* (**F12**). Cultures were exposed to the marketed products for either 5 or 15 minutes, after which relative numbers of live/dead cells were evaluated. Moxifloxacin was least cytotoxic (83.6%), with gatifloxacin (88.2%), levofloxacin (89.5%), and ofloxacin (90.9%) similar to one another, showing somewhat greater cytotoxicity, compared with moxifloxacin or the controls (Systane® [Alcon], 70.2%, and culture control, 70.9%). Matsumoto et al suggested that moxifloxacin inhibits *in vitro* corneal wound healing more than gatifloxacin, levofloxacin, and ofloxacin, with the order of inhibition ofloxacin ≈ levofloxacin < gatifloxacin < moxifloxacin << ciprofloxacin when tested at 0.6 mM (**F13**). In a similar study, Matsumoto et al reported ofloxacin inhibited *in vitro* corneal wound healing in the same model.[13] Gatifloxacin, ciprofloxacin, and moxifloxacin, at concentrations of 1 μg/mL or more, were all cytotoxic *in vitro* to human corneal endothelial cells and keratocytes (**F14**). Most of these studies used antibiotic solutions and not the commercial topical products. The presence of benzalkonium chloride in products, like Zymar, should result in a markedly different outcome in these cytotoxicity studies. *In vitro* assays may be useful in assessing comparative effects of drugs, or products, but may have limited relevance to clinical use.

The ocular surface is exposed to all components of the ophthalmic drug product, including excipients, and these may affect the cornea and conjunctiva. The antimicrobial preservative in these preparations has a great influence on ocular safety. All approved ophthalmic fluoroquinolone solutions are preserved with benzalkonium chloride, except moxifloxacin ophthalmic solution 0.5% and, recently, levofloxacin ophthalmic solution 1.5% (Iquix®, Vistakon), which have no designated preservative added. The safety of fluoroquinolones with respect to the cornea is of utmost importance, and the toxicological first principle that "the dose makes the poison" emphasizes the importance of safety to the tissues exposed to the highest concentration. Moxifloxacin ophthalmic solution achieves superior concentrations in corneal tissue and aqueous humor when administered topically, as compared with other fluoroquinolones, including gatifloxacin (**F15**).[16] This suggests the potential for a longer duration of drug concentrations above the MIC (Minimum Inhibitory Concentration) for infecting organisms (**F16, F17**). Rusinko et al reported that drug permeability and penetration correlate with lipophilicity and aqueous solubility for a series of seven fluoroquinolones (including moxifloxacin, gatifloxacin, and ofloxacin) (**F18**) and Owen et al demonstrated the superior permeability and penetration characteristics of moxifloxacin (**F19**). Corneal penetration of the drug was

[F11] Williams KK, McCartney MD, Rice RL, et al: The effects of moxifloxacin 0.5% ophthalmic solution or gatifloxacin 0.3% ophthalmic solution treatment on corneal wound healing in pigmented rabbits following anterior keratectomy. Cornea. Submitted for publication.

[F12] Yee RW, Sorour HM, Yee SB, et al: Comparison of relative toxicity of four ophthalmic antibiotics using the human cornea epithelial cell culture system (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4939, 2004.

[F13] Matsumoto S, Way W, Carlo K, Short B: Comparative toxicity of fluoroquinolones antibiotics on corneal cells *in vitro* (abstract). Am College Toxicol:17, 2003.

[F14] Skelnik DL, Clark LA, Bezwada P: Effect of drug concentration and exposure time of levofloxacin, ofloxacin, ciprofloxacin, gatifloxacin and moxifloxacin on human corneal endothelial cells and keratocytes (abstract). Invest Ophthalmol Vis Sci 44(Suppl):4739, 2003.

[F15] Robertson SM, Sanders M, Jasheway D, et al: Absorption and distribution of moxifloxacin, ofloxacin and gatifloxacin into ocular tissues and plasma following topical ocular administration to pigmented rabbits (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4906, 2004.

[F16] Levine J, Noecker R, Herrygers L, Clark T: Aqueous levels of moxifloxacin and gatifloxacin following different pre- and post-operative topical dosing protocols (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4911, 2004.

[F17] Mather R, Stewart JM, Prabriputaloong T, et al: Corneal concentrations of moxifloxacin following topical administration in a rabbit model (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4989, 2004.

much greater and more rapid for moxifloxacin ophthalmic solution 0.5% (91×10^{-7} cm/second; lag time, 49 minutes) than for gatifloxacin ophthalmic solution 0.3% (25×10^{-7} cm/second; lag time, 99 minutes), whereas corneal permeability to carboxyfluorescein was much greater after gatifloxacin ophthalmic solution 0.3% exposure (3.6 pM/mL/minute) than after moxifloxacin ophthalmic solution 0.5% (2.1 pM/mL/minute). Carboxyfluorescein does not normally cross the cornea but is an indicator of corneal permeability through epithelial cell junction disruption, as it readily passes through the disrupted epithelium. Thus, the superior penetration profile of moxifloxacin, nearly four times more, is attributable to the inherent physicochemical properties of the drug and not to disruption of tight junctions in the corneal epithelium. The carboxyfluorescein findings with gatifloxacin ophthalmic solution 0.3% are consistent with those of benzalkonium chloride–containing formulations, where the permeability is increased in a concentration-dependent manner. Benzalkonium chloride concentrations as low as 0.005% applied to the rabbit eye (15 times at 5-minute intervals) caused superficial epithelial swelling and desquamation.[10]

Clinical Relevance of Animal and *In Vitro* Findings

In vitro and animal studies in intact corneas consistently show that topical ophthalmic fluoroquinolones are safe even at very high concentrations and treatment regimens, and this seems consistent with clinical experience. Nguyen et al found no significant effects on conjunctival injection, chemosis, stinging/burning, lid thickness, ocular surface sensation, or pupil size after a single drop of moxifloxacin ophthalmic solution 0.5% to one eye and gatifloxacin ophthalmic solution 0.3% to the fellow eye of 10 normal subjects (**F20**). This was confirmed in a study of human ocular tolerability after a single drop of moxifloxacin ophthalmic solution 0.5%, in comparison with Tears Naturale Free (**F21**). However, Donnenfeld et al reported that normal young adult subjects receiving gatifloxacin ophthalmic solution 0.3% exhibited fewer increases in signs and symptoms of ocular intolerablity (i.e., conjunctival hyperemia, vascularity, ocular pain and irritation, and miosis) than those receiving moxifloxacin ophthalmic solution 0.5%.[4] The fluoroquinolone concentrations achieved in ocular tissues after topical instillation in animals and humans have been determined by a number of investigators and reviewed by Robertson et al.[16] The maximum concentrations achieved in the aqueous humor in rabbits ranged from 0.3 to 32.6 µg/mL depending on the dosage regimen and the fluoroquinolone. In humans, documented fluoroquinolone levels generally ranged from 0.11 to 2.28 µg/mL after topical treatment.[8] In those studies where various antibiotic products were compared, moxifloxacin generally achieved aqueous humor concentrations approximately 2 to 3 fold higher than gatifloxacin, ofloxacin or ciprofloxacin.[16] García-Sáenz et al reported aqueous humor concentrations of 2.33 µg/mL after 400 mg of moxifloxacin taken orally.[7] No reports of human cornea drug levels are available, but concentrations of moxifloxacin in rabbit cornea were 12.5 µg/g and 21.3 µg/g after a single dose or a 3-day t.i.d. regimen, respectively, as compared with 6.02 and 8.01 µg/g concentrations of ofloxacin by the same regimens and 4.85 µg/g for gatifloxacin t.i.d. (**F15**). Mather et al reported a mean rabbit cornea concentration of 158.66 µg/g at 60 minutes after topical dosing at 0, 5, 10, 60, 65, and 70 minutes (**F17**). In rabbit studies, drug concentrations in the cornea were found to be approximately 10–20 times those measured in aqueous humor, and if a similar relationship is present in humans, then cornea concentrations would be proportionately higher.

Corneal epithelial and endothelial cell morphologies were unchanged in volunteers receiving moxifloxacin ophthalmic solution 0.5% four times a day for 3 days after cataract surgery (**F22**). Moxifloxacin ophthalmic solution 0.5% and gatifloxacin ophthalmic solution 0.3% were tested on patients after laser-assisted *in situ* keratomileusis or laser epithelial keratomileusis surgeries had no effect on ophthalmic measures including quality of vision, comfort, and rates of corneal re-epithelialization.[5] Despite high tissue concentrations, no effects on postoperative corneal and conjunctival healing were reported. Corneal

F18 Rusinko A, May J, Liao J, et al: A study of the enhanced corneal penetration of moxifloxacin (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4907, 2004.

F19 Owen GR, Dembinska O, Stout KR, Mendiola MK: Corneal penetration and changes in corneal permeability of moxifloxacin versus gatifloxacin (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4910, 2004.

F20 Nguyen QH, Friedlaender MH, Sharf L, Breshears D: Objective and subjective measurement of drug toxicity (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4937, 2004.

F21 Wagner RS, D'Arienzo PA, Hallas SJ, et al: A comparative study in a normal pediatric population of the relative comfort of moxifloxacin 0.5% ophthalmic solution versus a tear substitute (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4913, 2004.

F22 Donaldson KE, Marangon FB, Schatz L, et al: Confocal analysis of the effects of moxifloxacin on the normal human cornea. Poster presented at Am Soc Cataract Refract Surg, May 1–5, 2004; San Diego CA.

epithelial healing time following photorefractive keratectomy was found to be better for subjects using moxifloxacin ophthalmic solution, 0.5% than for gatifloxacin ophthalmic solution, 0.3%, when given post-operatively, four times a day. Corneal epithelial defects had healed for 80% of eyes treated with moxifloxacin ophthalmic solution, 0.5%, as compared with 69% for gatifloxacin ophthalmic solution, 0.3% post-operative day 4. Epithelial defect size was also statistically significantly smaller for the moxifloxacin-treated eyes at day 4.[1] Thus, clinical experience shows that moxifloxacin ophthalmic solution 0.5% and other ophthalmic fluoroquinolones are safe and well tolerated in normal and postsurgical eyes.

Discussion

Fluoroquinolone antibiotics have proven safe and effective in both systemic and ophthalmic use. Nonclinical studies support the use of the ophthalmic products for the currently approved indications. Moxifloxacin is a fourth-generation fluoroquinolone with favorable characteristics such as potent efficacy and good ocular penetration. Systemic exposure (i.e., plasma drug levels) with topically applied fluoroquinolones is negligible, so untoward systemic effects are unlikely.

Nonclinical studies have established a high safety margin for ophthalmic moxifloxacin. Moxifloxacin ophthalmic solution given topically to rabbit eyes at concentrations of up to 3%, two drops four times a day for as long as 1 month, resulted in no significant ocular effects, including on corneal thickness, assessment by slit-lamp biomicroscopic or indirect ophthalmoscopy, and ocular histology. No systemic effects were evident as assessed by detailed in-life observations, clinical laboratory evaluations, and general histopathologic examinations. Extremely high topical doses of moxifloxacin ophthalmic solution instilled in normal monkey eyes for 3 months produced no ocular toxicity, with no abnormalities observed in corneal epithelium, stroma, or endothelium, and no significant systemic findings.

Some nonclinical *in vivo* studies have suggested potential effects of topical treatment with fluoroquinolones on healing rates in wounded cornea and differences between moxifloxacin and gatifloxacin, whereas other studies show similar wound healing, comparable with controls for both drugs. In general, it appears that there are no significant differences between the two drugs, and both drugs are safe for use under those conditions. *In vitro* studies of fluoroquinolones with human or rabbit corneal cells suggest that moxifloxacin has a low potential for dose- and time-dependent cytotoxicity.

Although some nonclinical studies have demonstrated inconsistent results with moxifloxacin ophthalmic solution 0.5% in the wounded cornea or wound healing, clinical reports consistently find no significant effects on postsurgical recovery with post-cataract, post–laser-assisted *in situ* keratomileusis, and post–laser epithelial keratomileusis use. Clinical studies have demonstrated high concentrations of moxifloxacin in aqueous humor after topical dosing. These high drug levels assure clinical efficacy, with concentrations of 10 to 30-fold higher moxifloxacin's MICs for common ocular pathogens.[18] Moxifloxacin ophthalmic solution 0.5% combines the therapeutic benefits of high antimicrobial efficacy and ocular tissue penetration with a favorable safety profile.

Method of Literature Search

A search of the MEDLINE and Toxline databases (1970–2005) was conducted using an online search tool (Endnote 5.0, ISI Researchsoft, Berkeley, CA). Search terms employed were *moxifloxacin, fluoroquinolone, ocular toxicity, ophthalmic toxicity,* and *eye toxicity.* All entries considered to be of significance were utilized, including those in the non-English literature if an English abstract was available. The reference section of each article was reviewed, and if it was felt to be of significance by adding additional data or refuting existing information, it was included. When necessary, data were computed from graphs and tables.

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ELSEVIER

Clinical Safety of Moxifloxacin Ophthalmic Solution 0.5% (VIGAMOX®) in Pediatric and Nonpediatric Patients With Bacterial Conjunctivitis

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Abstract. Five independent, multicentered, double-masked, parallel, controlled studies were conducted to determine the safety of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®) in pediatric and nonpediatric patients with bacterial conjunctivitis. Patients were randomized into one of two treatment groups in each study and received either moxifloxacin ophthalmic solution 0.5% b.i.d. or t.i.d. or a comparator. A total of 1,978 patients (918 pediatric and 1,060 nonpediatric) was evaluable for safety. The most frequent adverse event in the overall safety population was transient ocular discomfort, occurring at an incidence of 2.8%, which was similar to that observed with the vehicle. No treatment-related changes in ocular signs or visual acuity were observed with moxifloxacin ophthalmic solution 0.5%, except for one clinically relevant change in visual acuity. Thus, based upon a review of adverse events and an assessment of ocular parameters, moxifloxacin ophthalmic solution 0.5% formulated without the preservative, benzalkonium chloride, is safe and well tolerated in pediatric (3 days–17 years of age) and nonpediatric (18–93 years) patients with bacterial conjunctivitis. (**Surv Ophthalmol 50:S55–S63, 2005.**

Key words. adverse events • conjunctivitis • fluoroquinolone • moxifloxacin • ophthalmic • pediatric • safety • VIGAMOX®

Bacterial conjunctivitis is one of the most common ocular infections contracted by children; thus, the safety of a drug to treat ocular infections in children and in the overall population is of paramount importance.[4,5] Topical ocular fluoroquinolones, such as ciprofloxacin, have been used to safely treat conjunctivitis in pediatric and nonpediatric patients for many years.[7] Unfortunately, emerging bacterial resistance raises concerns about the effectiveness of current fluoroquinolones.[2,6,8] A solution of a new fourth-generation fluoroquinolone, moxifloxacin hydrochloride, is currently available and indicated for the treatment of bacterial conjunctivitis in patients 1 year and older.[9] Second- and third-generation fluoroquinolones target the enzymes deoxyribonucleic acid gyrase or topoisomerase IV to elicit their antibactericidal effect.[1] Unlike these earlier fluoroquinolones, the fourth-generation fluoroquinolones bind to both deoxyribonucleic acid gyrase and topoisomerase IV to provide a simultaneous attack on two different bacterial enzymes, thus requiring two independent mutations in the bacteria to develop resistance.[10]

Patient compliance with dosing of a topical antibiotic is necessary to effectively fight bacterial ocular

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infections. To enhance compliance, the currently marketed solution of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX) is formulated at a physiological pH of 6.8 and does not include the preservative, benzalkonium chloride, which can have toxic effects.[11] Unlike other topical ophthalmic antibiotics, moxifloxacin has been formulated and packaged as a self-preserved formulation and requires no added preservative agent (e.g., benzalkonium chloride). Thus, establishing the safety of ophthalmic moxifloxacin in pediatric and nonpediatric patients enhances the clinical success of this fluoroquinolone in its self-preserved formulation. Church et al reviewed the clinical safety profile of moxifloxacin when administered systemically and found that it was safe and well tolerated in the treatment of respiratory tract infections.[3]

Five independent phase II and phase III multicenter, double-masked, parallel, well-controlled studies were conducted to evaluate the safety and efficacy of moxifloxacin ophthalmic solution 0.5%. The safety data were integrated across these five clinical trials and are presented in Tables 1–4. The discussion of the data focuses on a comparison of the integrated data for moxifloxacin ophthalmic solution 0.5% with that of the vehicle because the number of patients receiving ofloxacin ophthalmic solution 0.3% (Ocuflox®, Allergan, Irvine, CA) and ciprofloxacin ophthalmic solution 0.3% (Ciloxan®, Alcon Laboratories) was significantly smaller and not evenly distributed between pediatric and nonpediatric patients.

Methods

STUDY DESIGN

Five clinical trials (phase II and phase III) were conducted to evaluate the efficacy and safety of a solution of moxifloxacin ophthalmic solution 0.5%. All studies were multicenter, double-masked, randomized, parallel, and controlled (active or vehicle). Patients of any race and either sex who were diagnosed with bacterial conjunctivitis were enrolled in the study; the minimum age requirement for each study varied, depending on the study design (Table 5). The primary efficacy endpoint was the clinical cure rate for bulbar conjunctival injection and conjunctival discharge. An institutional review board approved each study before its initiation.

Patients with any of the following conditions were excluded from study participation: contact lens wear

TABLE 1

Most Frequent Adverse Events in Pediatric versus Nonpediatric Patients*

	Moxifloxacin 0.5% b.i.d. or t.i.d.						Vehicle b.i.d. or t.i.d.					
	Pediatrics (≤17 Years) (N = 462)		Nonpediatrics (≥18 Years) (N = 531)		Total (N = 993)		Pediatrics (≤17 Years) (N = 323)		Nonpediatrics (≥18 Years) (N = 283)		Total (N = 606)	
Adverse Event	N	%	N	%	N	%	N	%	N	%	N	%
Ocular												
Ocular discomfort	9	1.9	19	3.6	28	2.8	7	2.2	6	2.1	13	2.1
Keratitis	3	0.6	5	0.9	8	0.8	2	0.6	6	2.1	8	1.3
Conjunctivitis	5	1.1	5	0.9	10	1.0	6	1.9	2	0.7	8	1.3
Ocular pruritus	3	0.6	9	1.7	12	1.2	1	0.3	4	1.4	5	0.8
Visual acuity decrease	2	0.4	6	1.1	8	0.8	3	0.9	5	1.8	8	1.3
Subconjunctival hemorrhage	4	0.9	2	0.4	6	0.6	2	0.6	2	0.7	4	0.7
Blepharitis	2	0.4	4	0.8	6	0.6	1	0.3	2	0.7	3	0.5
Nonocular												
Infection	12	2.6	8	1.5	20	2.0	15	4.6	1	0.4	16	2.6
Headache	3	0.6	9	1.7	12	1.2	3	0.9	16	5.7	19	3.1
Fever	10	2.2	0	0.0	10	1.0	5	1.5	1	0.4	6	1.0
Cold syndrome	3	0.6	3	0.6	6	0.6	2	0.6	2	0.7	4	0.7
Vomiting	6	1.3	0	0.0	6	0.6	5	1.5	4	1.4	9	1.5
Increased cough†	15	3.2	3	0.6	18	1.8	9	2.8	5	1.8	14	2.3
Rhinitis	11	2.4	3	0.6	14	1.4	11	3.4	4	1.4	15	2.5
Pharyngitis	4	0.9	4	0.8	8	0.8	7	2.2	6	2.1	13	2.1
Otitis media	14	3.0	1	0.2	15	1.5	10	3.1	1	0.4	11	1.8

* Occurring at an incidence of >5 reports (0.5%) in the moxifloxacin total column. Table includes related and non-related adverse events.

† Worsening of existing cough or development of a cough. The COSTART dictionary does not have a separate code for the development of a cough.

TABLE 2

Most Frequent Related Adverse Events*

	Moxifloxacin 0.5% b.i.d. or t.i.d.						Vehicle b.i.d. or t.i.d.					
	Pediatrics (≤17 Years) (N = 462)		Nonpediatrics (≥18 Years) (N = 531)		Total (N = 993)		Pediatrics (≤17 Years) (N = 323)		Nonpediatrics (≥18 Years) (N = 283)		Total (N = 606)	
Adverse Event	N	%	N	%	N	%	N	%	N	%	N	%
Ocular												
Ocular discomfort	9	1.9	18	3.4	27	2.7	5	1.5	5	1.8	10	1.7
Ocular pruritus	2	0.4	4	0.8	6	0.6	1	0.3	1	0.4	2	0.3
Ocular hyperemia	3	0.6	0	0.0	3	0.3	0	0.0	0	0.0	0	0.0
Ocular pain	0	0.0	3	0.6	3	0.3	0	0.0	0	0.0	0	0.0
Nonocular												
Headache	1	0.2	1	0.2	2	0.2	0	0.0	2	0.7	2	0.3
Taste perversion	1	0.2	3	0.6	4	0.4	0	0.0	0	0.0	0	0.0

* Occurring at an incidence of >1 report (0.1%) in the moxifloxacin total column. A generous cutoff was chosen due to the low frequency of related adverse events.

during the study; vision not correctable to 0.6 logarithm of the minimum angle of resolution (logMAR) or better (children younger than 3 years must be able to fix and follow); abnormal findings on fundus examination or presence of active inflammation in the cornea, iris, or anterior chamber at the day 1 visit; an allergy or hypersensitivity to fluoroquinolones or benzalkonium chloride; any current immunosuppressive disorder; or a suspected fungal, viral, or *Acanthamoeba* infection. In addition, patients who had any systemic or ocular disorder, complicating factors, or structural abnormality that would affect the study outcome were excluded from participation. Patients taking any of the following medications were also excluded from participation: any preserved topical ocular medications; a topical ocular antibacterial within 24 hours of the study; an oral antibacterial within 72 hours of the study; a systemic steroid within 14 days of the study; a systemic nonsteroidal anti-inflammatory within 24 hours of the study (unless it was a steady regimen continuing for at least 2 months); or any other investigational study medication or immunosuppressive therapy (including chemotherapy). Also excluded from the study were those women who were pregnant or nursing. Females of childbearing potential could participate if they had a negative urine pregnancy test before randomization and used adequate birth control throughout the study.

Patients satisfying the exclusion and inclusion criteria rated their ocular symptoms on a four-point scale, and investigators assessed the ocular signs of the patient. Each patient underwent an ophthalmic examination that may have included a measurement of visual acuity and an assessment of ocular signs. Not all studies measured visual acuity or ocular signs (Table 5). Up to three microbiological specimens were collected from the affected eye(s) of the patient. Finally, a dilated or undilated fundus examination was conducted for each patient, except in study C-01-34. All patients (or their legal guardians) gave written informed consent before study participation.

TABLE 3

Additional Safety Assessments: Visual Acuity and Changes in Ocular Signs

	Clinically Relevant Changes from Baseline to Any Visit				
	LogMAR or Snellen Visual Acuity		Changes in Ocular Signs		
Treatment	Total N	≥3 Line Decrease [N (%)]	Total N	Cornea [N (%)]	Iris/Anterior Chamber [N (%)]
Moxifloxacin 0.5%	737	7 (0.9)	807	4 (0.5)	0
Ofloxacin 0.3%	269	0	277	8* (2.9)	1 (0.4)
Vehicle	448	5 (1.1)	535	5 (0.9)	2 (0.4)

logMAR = logarithm of the minimum angle of resolution.

Visual acuity assessed in C-00-02, C-00-46, C-00-55, and C-01-66. Ocular signs evaluated in C-00-46, C-00-55, and C-01-66. Thus, no data for visual acuity and ocular signs are available for ciprofloxacin 0.3%. Patients 3 years and younger were excluded from visual acuity assessments.

* One person receiving ofloxacin 0.3% experienced a treatment-related change in the cornea.

TABLE 4

Most Frequent Adverse Events by Age*

	Moxifloxacin 0.5% (t.i.d. or b.i.d.)					
Adverse Event	Newborns < 28 Days (N = 100) [N (%)]	Infants and Toddlers 28 Days 23 Months (N = 66) [N (%)]	Children 2–11 Years (N = 237) [N (%)]	Adolescents 12–17 Years (N = 59) [N (%)]	Adults 18–64 Years (N = 477) [N (%)]	Elderly ≥ 65 Years (N = 54) [N (%)]
Ocular						
Blepharitis	0	0	1 (0.4)	1 (1.7)	4 (0.8)	0
Conjunctivitis	0	1 (1.5)	3 (1.3)	1 (1.7)	5 (1.0)	0
Dry eye	0	0	0	0	4 (0.8)	0
Keratitis	0	0	1 (0.4)	2 (3.4)	5 (1.0)	0
Ocular discomfort	0	0	7 (3.0)	2 (3.4)	19 (4.0)	0
Ocular pain	0	0	0	0	5 (1.0)	0
Ocular pruritus	0	0	2 (0.8)	1 (1.7)	9 (1.9)	0
Visual acuity decrease	0	0	2 (0.8)	0	5 (1.0)	1 (1.9)
Nonocular						
Fever	1 (1.0)	4 (6.1)	5 (2.1)	0	0	0
Headache	0	0	0	3 (5.1)	9 (1.9)	0
Increased cough†	1 (1.0)	3 (4.5)	11 (4.6)	0	3 (0.6)	0
Infection	0	2 (3.0)	10 (4.2)	0	8 (1.7)	0
Otitis media	0	5 (7.6)	9 (3.8)	0	1 (0.2)	0
Pharyngitis	0	0	2 (0.8)	2 (3.4)	4 (0.8)	0
Rhinitis	2 (2.0)	3 (4.5)	6 (2.5)	0	3 (0.6)	0

Adverse Events listed alphabetically.

* Four or more reports occurring in at least one age group. A more generous cutoff was chosen due to the smaller Ns in the age groups relative to Table 1. Table includes related and non-related adverse events.

† Worsening of existing cough or development of a cough. The COSTART dictionary does not have a separate code for the development of a cough.

After the screening examination, qualified patients were randomized to receive either moxifloxacin ophthalmic solution 0.5% or a comparator (i.e., vehicle, ofloxacin ophthalmic solution 0.3%, or ciprofloxacin ophthalmic solution 0.3%). Study C-00-46 compared moxifloxacin ophthalmic solution 0.5% with ofloxacin ophthalmic solution 0.3%, and study C-01-34 compared moxifloxacin ophthalmic solution 0.5% with ciprofloxacin ophthalmic solution 0.3%, whereas the other three studies were vehicle controlled (Table 5). Study medication was first administered by a member of the investigator's staff at the conclusion of the eligibility visit (day 1). In the phase III studies (C-00-46, C-00-55, C-01-34, and C-01-66), after the day 1 visit patients were instructed to administer one drop of the masked medication

TABLE 5

Designs of Clinical Studies Including Measured Safety Parameters

	C-00-02	C-00-46	C-00-55	C-01-34	C-01-66
Age range	1–89 years	1–85 years	1 month–89 years	2–80 days	48 days–93 years
Duration of treatment	3 days	4 days	4 days	4 days	4 days
Duration of assessment*	7 days	9 days	9 days	9 days	9 days
Moxifloxacin group	0.5% b.i.d.	0.5% t.i.d.	0.5% t.i.d.	0.5% t.i.d.	0.5% t.i.d.
Comparator group	Vehicle b.i.d.	Ofloxacin 0.3% q.i.d.	Vehicle t.i.d.	Ciprofloxacin 0.3% t.i.d.	Vehicle t.i.d.
No. of study sites	20	15	32	32	31
Location of study	USA	India	USA	USA	USA
Adverse events	Yes	Yes	Yes	Yes	Yes
Visual acuity	Yes	Yes	Yes	No	Yes
Ocular signs	No†	Yes	Yes	No	Yes

* Total days of enrollment in the study.

† In this study, assessment of ocular signs was an efficacy parameter only. In subsequent studies, the measurement of ocular signs was expanded to include safety assessments of the cornea and iris/anterior chamber.

in the conjunctival sac of both eyes t.i.d. for 4 days' total exposure. Patients were observed for a total of 9 days. In the phase II study, C-00-02, patients received either one or two drops of medication b.i.d. for 3 days' total exposure, and the patients were observed for a total of 7 days. A summary of the demographics of the patients integrated across the five studies is shown in Table 6.

SAFETY ASSESSMENTS

Safety data were pooled across the five clinical studies. The evaluation of safety was conducted on all patients who were randomized into the study and received at least one dose of the study drug. Adverse events were defined as any change (expected or unexpected) in a patient's ophthalmic and/or systemic health that occurred after initiation of study treatment. These changes included any changes in visual acuity or ocular signs as defined in each study protocol. The principal investigator and a medical monitor independently assessed causality of these events, and the events were coded using a modified COSTART dictionary.

The principal investigator assessed best corrected visual acuity at the day 1 (baseline) visit and at all subsequent visits for patients older than 3 years. In study C-01-34, visual acuity was not measured, as the study involved patients younger than 1 month. For all other patients, the logMAR (or Snellen) procedure was used to assess visual acuity. The maximum change in visual acuity for the worse eye in each patient (i.e., the eye with greatest decrease in visual acuity) was calculated as the change in logMAR lines (0.1 units = 1 logMAR line) from baseline to any visit. For pediatric patients whose visual acuity could not be determined using logMAR (or Snellen), an age-appropriate measurement method, either HOTV or Crowded Symbols, was used to calculate the maximum change in visual acuity. Clinically relevant changes in visual acuity were defined as a decrease of 3 or more logMAR (or Snellen) lines from baseline and resulted in the reporting of an adverse event.

Ocular signs were assessed in studies C-00-46, C-00-55, and C-01-66. Ocular signs (cornea and iris/anterior chamber) were assessed by the principal investigator using a penlight, ophthalmoscope, or slit lamp at day 1 (baseline) and at all subsequent (scheduled or unscheduled) visits. Clinically relevant changes in ocular signs were defined as any increase in parameter score from baseline (Table 7) and resulted in the reporting of an adverse event.

Results

OVERALL SAFETY POPULATION

The safety profile of moxifloxacin ophthalmic solution 0.5% was evaluated in 1,978 patients across five

TABLE 6

Demographics of Safety Population

	Moxifloxacin 0.5% (N = 993)		Ofloxacin 0.3% (N = 277)		Ciprofloxacin 0.3% (N = 102)		Vehicle (N = 606)	
Demographic Parameter	N	%	N	%	N	%	N	%
Sex								
Male	495	49.8	188	67.9	57	55.9	256	42.2
Female	498	50.2	89	32.1	45	44.1	350	57.8
Race								
White	500	50.4	0	0	70	68.6	409	67.5
Black	51	5.1	0	0	3	2.9	53	8.7
Other*	442	44.5	277	100.0	29	28.4	144	23.8
Iris color†								
Brown	321	32.3	276	99.6	34	33.3	300	49.5
Blue	224	22.6	0	0	48	47.1	171	28.2
Other	446	44.9	1	0.4	20	19.6	135	22.3
Age								
Newborn infants (<28 days)	100	10.1	0	0	97	95.1	0	0
Infants and toddlers (28 days–23 months)	66	6.6	4	1.4	5	4.9	63	10.4
Children (2–11 years)	237	23.9	21	7.6	0	0	222	36.6
Adolescents (12–17 years)	59	5.9	6	2.2	0	0	38	6.3
Adults (18–64 years)	477	48.0	220	79.4	0	0	260	42.9
Elderly (≥65 years)	54	5.4	26	9.4	0	0	23	3.8

* Moxifloxacin 0.5% other includes 290 Asian, 129 Hispanic, and 23 other. Ofloxacin 0.3% other includes 277 Asian. Ciprofloxacin 0.3% other includes 26 Hispanic and 3 other. Vehicle other includes 13 Asian, 118 Hispanic, and 13 other.

† Two patients in the moxifloxacin 0.5% treatment group have missing iris color data.

TABLE 7

Scoring of Ocular Signs Parameters

	Specifications	Definition of Terms	
		Normal (0)	Abnormal (1)
Cornea	Includes all corneal layers	Absence of active inflammation or active structural change	Presence of active inflammation or active structural change including focal scarring and fine deposition
Iris/anterior chamber	Includes evaluation of anterior chamber and its surrounding structure	Absence of active inflammation	Presence of active inflammation

clinical studies who received at least one dose of study medication. Moxifloxacin ophthalmic solution 0.5% was safe and well tolerated in the overall patient population. No serious adverse events assessed as related to therapy were reported. Overall, adverse events assessed as related to moxifloxacin ophthalmic solution 0.5% occurred in 4.7% of the patients, whereas 2.6% of the patients experienced adverse events assessed as related to the vehicle (data not shown). Adverse events in the moxifloxacin ophthalmic solution 0.5% and vehicle groups that were related to study therapy were generally mild in intensity and usually resolved on their own or with treatment. In addition, adverse events in the active-controlled studies (Table 8) were similar when comparing moxifloxacin ophthalmic solution 0.5% with ofloxacin ophthalmic solution 0.3% or with ciprofloxacin ophthalmic solution 0.3%.

As presented in Table 1, the most frequent adverse event (related and not related combined) in the overall safety population receiving moxifloxacin ophthalmic solution 0.5% was ocular discomfort (i.e., transient burning and stinging), which occurred at an incidence of 2.8% and was similar to that observed with the vehicle group (2.1%). In addition, transient ocular discomfort was the most common treatment-related event (Table 2) in both the moxifloxacin ophthalmic solution 0.5% group (2.7%) and the vehicle group (1.7%). These events of ocular discomfort were mostly mild in intensity, usually resolved without treatment, and rarely prevented a patient from completing the study.

The most frequent nonocular adverse event (related and not related combined) in patients receiving moxifloxacin ophthalmic solution 0.5% was general infection (Table 1), which occurred in 2.0% of the

TABLE 8

Most Frequent Adverse Events in the Active-Controlled Studies (C-00-46 and C-01-34)*

	C-00-46†				C-01-34‡			
	Moxifloxacin 0.5% t.i.d. (N = 277)		Ofloxacin 0.3% q.i.d. (N = 277)		Moxifloxacin 0.5% t.i.d. (N = 107)		Ciprofloxacin 0.3% t.i.d. (N = 102)	
Adverse Event	N	%	N	%	N	%	N	%
Ocular								
Ocular discomfort	4	1.4	1	0.4	0	0.0	0	0.0
Keratitis	3	1.1	3	1.1	0	0.0	0	0.0
Corneal infiltrate	2	0.7	3	1.1	0	0.0	0	0.0
Tearing	0	0.0	0	0.0	3	2.8	2	2.0
Ocular hyperemia	1	0.4	0	0.0	2	1.9	1	1.0
Nonocular								
Rash	0	0.0	0	0.0	3	2.8	3	2.9
Rhinitis	0	0.0	1	0.4	2	1.9	4	3.9
Surgical/medical procedure§	0	0.0	0	0.0	2	1.9	3	2.9
Eructation	0	0.0	0	0.0	2	1.9	0	0.0

* Occurring at an incidence of >1 report in either moxifloxacin column. A generous cutoff was chosen due to the low frequency of adverse events in the active-controlled studies. Table includes related and non-related adverse events.

† Most patients were nonpediatric patients.

‡ All patients were pediatric patients (2–30 days old).

§ For moxifloxacin 0.5%, 1 case of circumcision and 1 case of frenulectomy. For ciprofloxacin 0.3%, 3 cases of circumcision.

patients. This incidence of infection was similar to that seen with the vehicle (2.6%). These reported adverse events of infection were not related to the study drug (Table 2). Other frequently reported non-ocular adverse events (i.e., increased cough, rhinitis, and otitis media) were not related to moxifloxacin and occurred at a lower incidence than in the vehicle.

After an assessment of visual acuity, seven patients (an incidence of 0.9%) receiving moxifloxacin ophthalmic solution 0.5% exhibited a clinically relevant decrease in visual acuity (Table 3), which is similar to that for the vehicle (1.1%). Clinically relevant changes in visual acuity were defined as a decrease of 3 or more logMAR (or Snellen) lines from baseline (day 1 visit). No patient discontinued from the study due to a change in visual acuity. For patients exposed to moxifloxacin ophthalmic solution 0.5%, no changes in visual acuity were related to the study drug, with one exception. In this incident, a 4-year-old child was uncooperative in follow-up visual acuity measurements, and the event was conservatively reported as related. No clinically relevant-treatment-related changes in ocular signs (cornea or iris/anterior chamber) were observed with moxifloxacin ophthalmic solution 0.5%.

PEDIATRIC POPULATION

In the five clinical studies, the safety profile of a solution of moxifloxacin ophthalmic solution 0.5% was evaluated in 462 pediatric patients (3 days–17 years old) who received at least one dose of study medication. Moxifloxacin ophthalmic solution 0.5% was safe and well tolerated in pediatric patients, including all age categories: newborns (0–27 days), infants and toddlers (28 days–23 months), children (2–11 years), and adolescents (12–17 years). No serious adverse events assessed as related to therapy were reported in any pediatric patient. A small incidence of pediatric patients (3.5%) who received moxifloxacin ophthalmic solution 0.5% experienced a treatment-related adverse event, with a similar incidence of 2.8% observed for those receiving the vehicle (data not shown). Treatment-related adverse events experienced by pediatric patients were generally mild in intensity and usually resolved on their own.

As seen in the overall safety population, the most common ocular adverse event (related and not related combined) experienced by pediatric patients in the moxifloxacin ophthalmic solution 0.5% group was ocular discomfort (Table 1), which occurred in 1.9% of the patients, with a similar incidence of 2.2% reported for those in the vehicle group. All adverse events of ocular discomfort experienced by pediatric patients receiving moxifloxacin ophthalmic solution 0.5% were related to the study drug, whereas five of seven adverse events of discomfort for those in the vehicle group were treatment-related adverse events. Although ocular discomfort was the most common treatment-related adverse event, the events were generally mild, usually resolved on their own within minutes of onset, and did not interrupt patient participation in the study. Most ocular adverse events (related and not related combined) were not reported in more than three pediatric patients, except ocular discomfort, conjunctivitis, subconjunctival hemorrhage, and tearing.

The most common systemic adverse event (related and not related combined) experienced by pediatric patients in the moxifloxacin ophthalmic solution 0.5% and vehicle groups was increased cough (includes the development of a cough; see Table 1 footnote), which occurred in 3.2% and 2.8% of patients, respectively (Table 1). For patients exposed to moxifloxacin ophthalmic solution 0.5%, most of the frequently reported systemic adverse events (including increased cough, infection, rhinitis, and otitis media) were assessed as not related to the study drug.

NONPEDIATRIC POPULATION

In the five clinical studies, the safety profile of moxifloxacin ophthalmic solution 0.5% was evaluated in 531 nonpediatric (i.e., adult and elderly) patients who were 18 to 93 years old and received at least one dose of study medication. Moxifloxacin ophthalmic solution 0.5% was safe and well tolerated in adult (18–64 years old) and elderly (65 years and older) patients. No serious adverse event assessed as related to therapy was reported in any adult or elderly patient. Adult and elderly patients who received moxifloxacin ophthalmic solution 0.5% experienced treatment-related adverse events (5.8%) at an incidence similar to that of those who received vehicle (3.9%; data not shown).

As seen in the overall safety population, the most common adverse event experienced by adult and elderly patients exposed to moxifloxacin ophthalmic solution 0.5% was ocular discomfort, which occurred in 3.6% of the patients (Table 1) versus an incidence of 2.1% for patients receiving the vehicle. These events of ocular discomfort were mild in intensity (except for one incident that was moderate), resolved without treatment, and did not prevent any patient from completing the study with one exception. All events of ocular discomfort in the nonpediatric population were related to the study drug, with one exception in both the moxifloxacin ophthalmic solution 0.5% group and the vehicle group. Nonpediatric patients who complained of ocular discomfort in the moxifloxacin ophthalmic solution 0.5% group (Table 4) were only in the adult age group and not

the elderly group. Also, although visual acuity decrease was reported in 1.1% of the nonpediatric patients receiving moxifloxacin ophthalmic solution 0.5% (and at an incidence of 1.8% in the vehicle group), none of these were treatment-related. In fact, besides ocular discomfort, ocular pruritus, and ocular pain (Table 2), no treatment-related ocular adverse event was reported in more than one nonpediatric patient across all five studies.

Common systemic adverse events (related and not related combined) experienced by nonpediatric patients (Table 1) who received moxifloxacin ophthalmic solution 0.5% were general infection (1.5%) and headache (1.7%), which all occurred in adult patients (Table 4). With one exception of headache, these systemic events were not related to the study drug for patients exposed to moxifloxacin ophthalmic solution 0.5%. Nonpediatric patients exposed to the vehicle reported an incidence of 0.4% for infection and 5.7% for headache, which were mostly not related to the study drug.

Conclusion

Because the use of topical fourth-generation fluoroquinolones is relatively new, it is important to establish the safety and tolerability of moxifloxacin ophthalmic solution 0.5% formulated without benzalkonium chloride. Thus, the safety of moxifloxacin ophthalmic solution 0.5% was evaluated against a comparator (i.e., ofloxacin ophthalmic solution 0.3%, ciprofloxacin ophthalmic solution 0.3%, or vehicle) in clinical studies that included 1,978 patients pooled across five studies. No serious treatment-related adverse events were reported in these studies. In fact, no adverse event (treatment-related or not related) was reported across the five studies at an incidence greater than 3.0% in the moxifloxacin ophthalmic solution 0.5% group. Ocular adverse events in the overall population, particularly those related to the study drug, were generally mild in intensity and usually resolved without intervention. A review of the adverse event profile, visual acuity assessments, and ocular signs parameters for patients enrolled in the five clinical studies demonstrated that the safety and tolerability of a solution of moxifloxacin ophthalmic solution 0.5% was similar to that of the vehicle. In addition, no safety concerns were identified when comparing moxifloxacin ophthalmic solution 0.5% with ofloxacin ophthalmic solution 0.3% or ciprofloxacin ophthalmic solution 0.3%.

The most common treatment-related adverse event in the overall safety population, including both pediatric and nonpediatric patients, was transient ocular discomfort. Although a solution of moxifloxacin ophthalmic solution 0.5% is the highest concentration of a fourth-generation fluoroquinolone currently available, the incidence of transient discomfort in those receiving moxifloxacin ophthalmic solution 0.5% t.i.d. was similar to that seen in the vehicle group. These events of ocular discomfort were usually mild in intensity and generally resolved on their own after a few minutes. From product launch in May 2003 through December 31, 2004, only 12 postmarketing adverse event reports of eye irritation have been received out of more than 3.6 million units of moxifloxacin ophthalmic solution 0.5% (VIGAMOX®) sold, confirming the safety and tolerability of this product already demonstrated in the clinical studies. Besides transient ocular discomfort, ocular pruritus and taste perversion were the only treatment-related adverse events reported in more than three patients receiving moxifloxacin ophthalmic solution 0.5% in the overall safety population across the five clinical studies.

Because bacterial conjunctivitis is an infection commonly diagnosed in pediatric patients, the safety of moxifloxacin ophthalmic solution 0.5% was evaluated in individuals as young as 3 days.[4] The safety profile of moxifloxacin ophthalmic solution 0.5% in pediatric patients was similar to that observed in nonpediatric patients and in the overall safety population. In particular, ocular adverse events were detected in the pediatric patients, including newborns, at an incidence less than 2.5%, which demonstrates the safety and tolerability of moxifloxacin ophthalmic solution 0.5% even in the youngest pediatric patients. Some nonocular adverse events were reported at slightly higher incidences relative to ocular events; however, these events were generally not related to the study drug. In fact, besides ocular discomfort and ocular hyperemia (data not shown), no treatment-related adverse event occurred in more than two pediatric patients exposed to moxifloxacin ophthalmic solution 0.5% across the five clinical studies.

In summary, data integrated across five well-controlled clinical studies demonstrate that moxifloxacin ophthalmic solution 0.5% presents no safety concerns for patients, including newborns and infants/toddlers. In addition, the safety of moxifloxacin ophthalmic solution 0.5% did not significantly differ from that of ofloxacin ophthalmic solution 0.3%, ciprofloxacin ophthalmic solution 0.3%, or the vehicle. Thus, moxifloxacin ophthalmic solution 0.5% dosed t.i.d. is safe and well tolerated in pediatric and nonpediatric patients.

Methods of Literature Search

References cited in this article were identified from searches of the following computer-based databases:

MEDLINE, reference lists of review articles, and Association for Research in Vision and Ophthalmology abstracts (2001–2005). Key words employed were VIGAMOX®, *moxifloxacin, fluoroquinolone, pediatric, adverse events,* and *safety.* No non-English articles are cited.

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CONCLUSION

Future of Ophthalmic Anti-infective Therapy and the Role of Moxifloxacin Ophthalmic Solution 0.5% (VIGAMOX®)

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Abstract. The vintage antibiotics that were available in the 1950s–1980s were sometimes toxic, had limited spectra, and were bacteriostatic agents, and they have been replaced by significantly broader-spectrum therapies. We ask more of our future antibiotic products for ophthalmology: they must be 1) broad spectrum, 2) convenient to use, 3) useful prophylactically, 4) effective therapeutically, 5) benzalkonium chloride–free, 6) comfortable, and 7) nontoxic. The emergence of antibiotic resistance has focused us on more potent agents effective against resistant strains of bacteria. Fluoroquinolones have become a dominant family of ophthalmic antibiotics. But even the older fluoroquinolones (e.g., ofloxacin, ciprofloxacin) have lost much of their effectiveness against some important ocular isolates. Considering all of the characteristics for an ideal ophthalmic antibiotic product available today, moxifloxacin ophthalmic solution 0.5% represents a primary antibiotic product of choice for treating and preventing ophthalmic infections. (**Surv Ophthalmol 50:S64–S67, 2005.**

Key words. benzalkonium chloride • ciprofloxacin • fluoroquinolones • gatifloxacin • levofloxacin • moxifloxacin • ofloxacin • ophthalmic therapy • VIGAMOX®

Product Evolution and Demands

Gone are the days in the 1950s and 1960s when there was a wide range of choices for antibiotic therapy. Eye care practitioners no longer use sulfonamides, chloramphenicol, polymyxin, or bacitracin to treat ophthalmic infections. The use of aminoglycosides (neomycin, gentamicin, tobramycin) is waning as better agents in the fluoroquinolone family become available. These 1950s vintage antibiotics were sometimes toxic, had limited spectra, were bacteriostatic agents, and have been replaced by significantly broader-spectrum therapy. We ask more of our future antibiotic products for ophthalmology. They must be very effective against a wide range of infectious agents. They must be convenient to use for the surgeon and patient. They must cure quickly or sterilize the surgical eye area effectively. They should be able to be used prophylactically to prevent infections and therapeutically to cure infections. They must be able to be used in a wide variety of people, from neonates to geriatric patients. They must be broad enough to be used prophylactically and strong enough to cure serious, specific infections. Their dosage regimens must be simple, yet effective. They

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must be comfortable and nontoxic to the eye. They should be useful in treating or preventing a wide range of ocular infections (e.g., conjunctivitis, keratitis, endophthalmitis, blepharitis, dacryocystitis).

Growing Antibiotic Resistance

Growing microbial resistance to current antibacterial agents and widening gaps in antibiotic coverage create a need for a more potent and genetically smart fluoroquinolone. When ciprofloxacin, the first ocular fluoroquinolone, became available for ophthalmic use roughly a decade and a half ago, there was tremendous excitement. This was our knockout punch in the fight to prevent ocular infection, especially after cataract and refractive surgery. Today, however, our most impressive weapon has lost some of its punch.[13,16,24] Several microorganism groups have developed resistance to ciprofloxacin and its sister fluoroquinolones, ofloxacin and levofloxacin, more quickly than imagined, and resistance levels are increasing each year (F1).[16]

The bacteria hold most of the cards for the future. They will evolve and respond to their environment and produce progeny that will be resistant to today's antibiotics. Humans can only try to keep ahead of these clever creatures. Abandoning the old antibiotics and taking on the new is the only way to keep abreast of and even stop resistant trends. Continuing to use older, previous-generation antibiotics will only facilitate the continued development of resistant strains.[11] To our knowledge, there are no studies that prove or suggest topical application of moxifloxacin has the potential to induce microbial resistance distal to the site of instillation.

Conjunctivitis

Conjunctivitis occurs worldwide and affects people of all ages, all social strata, and both sexes. It has been cited as one of the most frequent causes of self-referral in the practice of comprehensive ophthalmology.[8,12,14,18] According to the American Academy of Ophthalmology (F2), conjunctivitis infrequently causes permanent visual loss or structural damage, but the economic impact of the disease in terms of lost work time, although undocumented, is doubtless considerable.

Fluoroquinolones

The fluoroquinolones are an evolving and powerful class of broad-spectrum antimicrobial agents used in the prevention and treatment of a variety of ocular infections; however, resistance to currently available agents in the class has been emerging among ocular pathogens.[2,4] They interfere with bacterial deoxyribonucleic acid synthesis, and newer generations of these compounds have improved broad-spectrum coverage. The topical fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin, were approved in 2003 by the US Food and Drug Administration for ocular indications. These antibiotics represent the most advanced group of compounds within the class, offer a unique dual-binding mechanism of action in gram-positive organisms, and have activity against otherwise resistant species.[4] Recent reports (F3) have indicated that the fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin, are more effective than earlier generations of fluoroquinolones and tobramycin, based on minimum inhibitory concentrations (MICs) and susceptibility results. Several recent *in vivo* studies using prophylactic models with rabbits have shown the potency of these antibiotics in preventing infections by common pathogens.[5,9,17] Although further clinical evidence of their efficacy in prophylaxis and treatment of human ocular infections is required, there is a growing need for compounds with this potential to combat emerging resistance.[4,6]

Benzalkonium Chloride–Free Products

Agents that are innately antibacterial, such as antibiotics, like the fluoroquinolones, have the opportunity of being formulated in multiple-dose containers without added antimicrobial preservative agents, such as benzalkonium chloride. This preservative has served the ophthalmic community well over the last 50 years and is still required for preserving antiglaucoma and other ophthalmic products. But researchers should take the opportunity to avoid additional chemicals in any ophthalmic formulation, if possible, unless new data suggest some previously unrecognized benefits. Moxifloxacin ophthalmic solution (VIGAMOX®, Alcon Laboratories, Inc., Fort Worth, TX) is the first fluoroquinolone antibiotic preparation available in the US that does not need benzalkonium chloride to be adequately preserved (F4). There are a number of benzalkonium chloride–free fluoroquinolone products for ophthalmology available in Japan.

F1 Alfonso EC: Why is the next generation of antibiotics so important? Refractive Eyecare Ophthalmol 7(Suppl):1, 2003.

F2 AAO, Cornea/External Disease Panel and the Preferred Practice Patterns Committee: Conjunctivitis. San Francisco, AAO, 2003.

F3 Tepedino ME: Microbiological analyses of the activity of 7 anti-infectives against isolates from patients with acute bacterial conjunctivitis (abstract). Invest Ophthalmol Vis Sci 45(Suppl): 4914, 2004.

F4 Schlech BA, Sutton S, Rosenthal RA, et al: Antimicrobial preservative effectiveness of VIGAMOX®™ (abstract). Invest Ophthalmol Vis Sci 45(Suppl):4913, 2004.

Therapeutic Usage

Topical therapy for treatment of infections remains an important and convenient avenue for the physician. The ability of an antibiotic to cure an ocular infection quickly and prevent serious vision loss is a paramount consideration for evaluating the effectiveness of therapeutic antibiotics. The ability of the antibiotic to penetrate the ocular tissues and kill and eradicate the pathogens at the site of the infection is an important goal. At this time, antibiotics such as moxifloxacin have better ocular penetration qualities than earlier fluoroquinolones, such as ciprofloxacin or ofloxacin (**F5, F6, F7**).[15,22]

Prophylactic Usage

The prevention of infections before, during, and after surgery and the use of prophylactic antibiotic products will undoubtedly continue in the future.[1,7,20,21,25] With this use, the antibiotic with the widest spectrum, lowest number of resistant strains, and fewest side effects should be the agent of choice for prophylaxis. The broad, shotgun approach still has merit in the surgical suite (**F8**).[10,17,23,25]

Antibiotic Susceptibility Testing and Breakpoints

The future of susceptibility testing is uncertain. The links between tests that define a pathogen as resistant or susceptible to a particular antibiotic are coming under fire. Standards have been set for systemic breakpoints for most antibiotics, but it is argued that these levels are not really relevant to the antibiotic levels achievable in ocular tissues via topical dosing.[19] The relatively poor predictive value of *in vitro* susceptibility is even dramatized more when systemic breakpoints are applied to ophthalmic antibiotics and their usage.[26] However, MICs are still of great value for rank ordering the power of various antibiotics or comparing the organisms that make up the most resistant or less sensitive groups.[5]

Culturing Bacterial Pathogens

Isolating and identifying an infecting organism is still a key principle of the 1883–1884 postulates defined by Robert Koch. Microbiologists will continue to show the virtue of culturing, isolating, or detecting the main pathogen in ophthalmic infections. It has been shown that culture confirmation affects the antibacterial therapeutic response rate of ulcerative keratitis.[28] Corneal infections by relatively ciprofloxacin-resistant bacteria respond more slowly to ciprofloxacin therapy. Antibacterial susceptibility testing of corneal cultures may predict the fluoroquinolone therapeutic response rate of bacterial keratitis.[27]

Ideal Antibiotic Product for Ophthalmology

For today's time and considering all of the characteristics for an ideal ophthalmic antibiotic product, moxifloxacin ophthalmic solution 0.5% (as VIGAMOX®) represents a primary antibiotic product of choice for treating and preventing ophthalmic infections. This includes improved effectiveness and potency, spectrum breadth, utility in treating and preventing infections, greater solubility, enhanced ocular penetration, acceptable safety, lack of benzalkonium chloride, and lower risk of resistance development. These virtues have been highlighted in this supplement. Nevertheless, the future will require even more advanced medications and therapy options. Such is the nature of infectious disease control for ophthalmology.

Method of Literature Search

We performed a literature search for this article based on MEDLINE database searches from 1990 to 2005, using varying combinations of the search terms *ocular infections, ophthalmic antibiotics, moxifloxacin, gatifloxacin, ciprofloxacin, ofloxacin, fluoroquinolones, therapy, prophylaxis,* and *future.* Relevant English journal articles and/or abstracts were selected for review.[6]

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Dr Schlech is an employee of Alcon Research, Ltd. Dr Blondeau has no proprietary or commercial interest in any product mentioned or concept discussed in the article. Dr Blondeau is a consultant for Allergan and Alcon and has received research grants from and is on the speaker bureau of numerous pharmaceutical companies, including Allergan and Alcon.

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Corneal Penetration Behavior of β-Blocking Agents I: Physicochemical Factors

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Abstract □ Rabbit corneas were excised and mounted in a chamber to determine the permeability characteristics of a group of β-blocking agents which varied in octanol–water partitioning over a fourfold logarithmic range. From the permeability rate at steady state, permeability coefficients (pH 7.65) were determined. For each drug the distribution coefficient and pK_a were measured, permitting the partition coefficients to be estimated. Various correlations were determined for the log permeability coefficient as a sum of log functions of the partition (or distribution) coefficient, molecular weight, and/or degree of ionization. The best fit, as judged by a high correlation coefficient ($r = 0.9756$) and lack of systematic deviation, was represented by: $\log P_T = 0.623 \log DC - 0.108(\log DC)^2 - 5.0268$.

Keyphrases □ β-Blocking agents—permeability characteristics, excised rabbit corneas, physicochemical factors □ Permeability—β-blocking agents, excised rabbit corneas, physicochemical factors □ Ophthalmic drugs—β-blocking agents, corneal permeability, rabbits, physicochemical factors

Whenever an ophthalmic drug is applied topically to the eye, only a small amount (<10%) actually penetrates the cornea and reaches the internal eye tissues (1–3). Precorneal factors, such as rapid drainage by the nasolacrimal apparatus and noncorneal absorption, account for the poor absorption (4). As a result, optimal absorption depends on achieving a rapid penetration rate across the cornea to minimize the competing, but nonabsorptive rate factors. Rapid penetration either permits a lower dose to be administered or, in the case of an inactive drug, leads to the development of a clinically effective drug.

The penetration potential of a drug with regard to its chemical structure can be assessed by the use of the partition coefficient of the drug. This has been shown for the cornea by Schoenwald and Ward (5) and by Mosher and Mikkelson (6). Schoenwald and Ward (5) determined the permeability rates across excised rabbit corneas for 11 steroids. Permeability coefficients for each steroid were calculated, and their logarithms were plotted against their respective log octanol–water partition coefficients. A parabolic relationship fit the data, with optimal permeability observed at a log partition coefficient of 2.9. Likewise, Mosher and Mikkelson (6) determined the *in vitro* corneal transport of *n*-alkyl-*p*-aminobenzoate ester homologues. For this series a parabolic equation also fit the data; optimal permeability was observed at a log partition coefficient of 2.5–2.6 (*n*-propyl homologue).

Although relative potency is a significant factor, a rapid penetration rate can contribute significantly to effectiveness. For example, prednisolone acetate (1% ophthalmic suspension) has been ranked as the most effective topical anti-inflammatory agent when the epithelium of the inflamed cornea is intact (7), whereas prednisolone (equally potent orally) is not effective topically. The prodrug di-

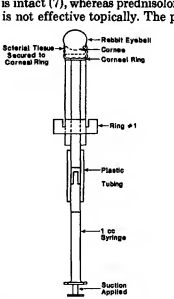

Figure 1—*Corneal holder for excised corneal preparation used in the permeability experiment.*

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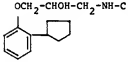
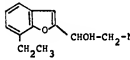
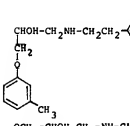
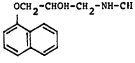
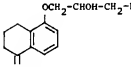
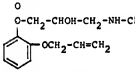
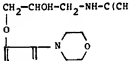
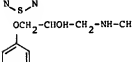
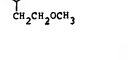
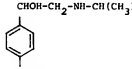
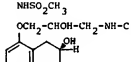
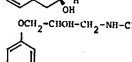
Table I—Chemical Structures, pK_a, and Partition Coefficients of β-Blocking Agents

Structure	Compound	pK_a	PC[a]
Very lipophilic:			
	Penbutolol	9.26	14,200.0
	Bufuralol	8.97	4,460.0
	Bevantolol	8.38	1010.0
	Propranolol	9.23	1640.0
Lipophilic:			
	Levbunolol	9.32	249.0
	Oxprenolol	9.32	235.0
	Timolol	9.21	82.0
	Metoprolol	9.24	76.0
Hydrophilic:			
	Acebutolol	9.20	59.0

continued

Table I—*Continued*

Structure	Compound	pK_a	PC[a]
Hydrophilic:			
	Sotalol	pK_{a1} = 8.15 pK_{a2} = 9.65	0.24
	Nadolol	9.39	8.5
	Atenolol	9.32	1.46

[a] Octanol–aqueous partition coefficient; the distribution coefficient was determined at pH 7.4 and 35° and converted through Eq. 2 to PC.

pivefrin is another example of a drug with improved corneal penetration when compared with the parent drug, epinephrine (8). A more rapid penetration rate for the prodrug has led to use of a reduced dosage and the observation of less ocular side effects.

The β-blocking agent timolol was introduced commercially to treat glaucoma following topical instillation of eye drops. Propranolol (9), atenolol (10), metoprolol (11), and practolol (10) also lower intraocular pressure, whereas nadolol and sotalol appear not to (12), even though nadolol is approximately equal in potency to propranolol. The purpose of this study was to compare the permeability of a series of β-blocking agents with a fourfold range in partitioning behavior across excised rabbit corneas to determine if optimal permeability can be identified.

EXPERIMENTAL

Drugs—β-Blocking agents used in the experiments included acebutolol hydrochloride[1], atenolol[2], beventolol hydrochloride[3], bufuralol hydrochloride[4], levbunolol hydrochloride[3], metoprolol tartrate[5], nadolol[6], oxyprenolol hydrochloride[4], penbutolol sulfate[7], propranolol hydrochloride[8], sotalol hydrochloride[9], and timolol maleate[10]. Structures of each drug are shown in Table I.

Potentiometric Titration Method for the Determination of pK_a—A pH meter[11] connected to a titrator[12] and equipped with a combination electrode[13] was used. The titrator was equipped with a 1.0-mL syringe-type buret and was used for all titrations in the study. The buret was attached to a delivery tip capable of accurately metering 0.005

[1] May & Baker LTD Research Laboratories.
[2] Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, Del.
[3] Warner-Lambert Company, Pharmaceutical Research Division, Ann Arbor, Mich.
[4] Roche Products LTD, Research Department.
[5] CIBA Pharmaceutical Co., Division of CIBA-GEIGY Corp., Summit, N.J.
[6] E. R. Squibb & Sons, Inc., Princeton, N.J.
[7] Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.
[8] Ayerst Laboratories, Inc., New York, N.Y.
[9] Mead Johnson & Company, Evansville, Ind.
[10] Merck Sharp & Dohme Research Lab, Division of Merck & Co., Inc., Rahway, N.J.
[11] Model 620, Fisher Accumet pH meter.
[12] Metrohm Multi-Dosimat E415, Herisau, Switzerland.
[13] Metrohm AG 9100, Herisau, Switzerland.

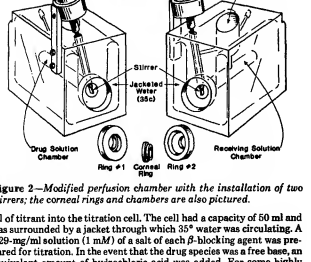


Figure 2—*Modified perfusion chamber with the installation of two stirrers; the corneal rings and chambers are also pictured.*

ml of titrant into the titration cell. The cell had a capacity of 50 ml and was surrounded by a jacket through which 35° water was circulating. A 0.29-mg/ml solution (1 mM) of a salt of each β-blocking agent was prepared for titration. In the event that the drug species was a free base, an equivalent amount of hydrochloric acid was added. For some highly lipophilic drugs, such as penbutolol and bufuralol, a concentration as low as 100 μg/ml was used to prevent precipitation during drug titration.

An aliquot (25–40 ml) of drug solution was accurately transferred to the titration cell, maintained at 35°. Nitrogen continuously flowed over the sample solution to prevent carbon dioxide absorption from the surrounding air. At each titration interval, a volume of 0.005–0.02 ml of titrant was added while stirring. For the majority of β-blocking agents, which have pK_a values of 8–9.4 at 35°, the titration usually started at pH ~6 and ended at pH 10 or 11. A modified Gran plot was used to determine the K_a for each compound (13) with the exception of sotalol, which contained two K_a values determined by the Speakman method (14).

Determination of Distribution Coefficients (15)—Sorensen's phosphate buffer (pH 7.38) was prepared from monobasic potassium phosphate and dibasic sodium phosphate. The buffer and octanol were mutually saturated at 35° before use. The distribution coefficient at 35° was determined by dissolving drug in the aqueous-buffer phase and shaking intermittently with octanol at 35° for 5 hr to reach a distribution equilibrium. The volume ratio of octanol and buffer depended on the lipophilicity of the drug. The volumes of each phase were chosen so that drug concentration in the aqueous phase, before and after extraction, could be measured by high-performance liquid chromatography (HPLC). Centrifugation was used to separate the two phases.

The distribution coefficient (DC) was calculated by:

$$DC = \frac{(C_b - C_a)V_w}{C_b V_o} \qquad \text{(Eq. 1)}$$

where C_b and C_a represent the concentrations in the aqueous-buffer phase before and after distribution, respectively; V_w represents the volume of the aqueous phase; and V_o, the volume of the octanol phase. The partition coefficient (PC) was calculated from the distribution coefficient by:

$$PC = DC\left(1 + \frac{1}{\text{antilog}\,(\text{pH} - \text{p}K_a)}\right) \qquad \text{(Eq. 2)}$$

The pH was measured from the buffered phase at 35° after distribution was complete. All distribution coefficients reported here were measured at pH 7.4, but through the use of Eq. 2 were converted to pH 7.65, the pH of the excised corneal experiments.

Excised Cornea Procedure—Male New Zealand White rabbits[14], weighing 1.6–2.0 kg each, were sacrificed by injecting a bolus of air into the marginal ear vein. The intact eye, along with the lids and conjunctival sac, was then enucleated. The exposed cornea of the enucleated eye was carefully placed on a corneal holder, which maintained the cornea curvature and held the eye in place (5, 16, 17). Various tissues of the eye were dissected leaving the cornea, a small ring of scleral tissue, and the palpebral conjunctiva, which was tied to the corneal ring (Fig. 1).

The conjunctival and scleral tissue served as a gasket and permitted the cornea to be suspended within the corneal ring, which was then positioned between rings 1 and 2 and placed in the center of the perfusion chamber. The chamber was made from acrylic plastic[15] and was jacketed to maintain the cornea and the perfusion solution at 35° (5, 16, 17).

Bicarbonated Ringer's solution was modified (17) to preserve tissue integrity of an excised cornea over 6 hr and used throughout the perfusion studies. It was prepared in two parts: Part I was composed of sodium chloride (12.4 g/liter), potassium chloride (0.716 g/liter), monobasic sodium phosphate monohydrate (0.206 g/liter), and sodium bicarbonate (4.908 g/liter); part II was composed of calcium chloride dihydrate (0.230 g/liter), magnesium chloride hexahydrate (0.318 g/liter), glucose (1.80 g/liter), and oxidized glutathione[16] (0.184 g/liter). Both parts were stored in the refrigerator and were used in ~3 weeks to prevent mold growth. Equal volumes of parts I and II were mixed prior to use.

Within 20–40 min of death, the cornea was mounted and clamped between two cylindrical compartments of the perfusion chamber. A measured volume (7.0 ml) of bicarbonated Ringer's solution was added first to the endothelial side as the receiving solution to prevent the cornea from buckling. An equal volume of solution containing a β-blocking agent was then added to the epithelial side as the drug solution. The perfusion chamber system was designed in such a way that the height of the receiving solution was slightly higher than that of the drug solution to ensure that the cornea would not buckle during the course of the experiment. A mixture of O_2–CO_2 (95:5) was bubbled through the fluids in both chambers for 10 min to achieve a pH of 7.65 before being added to the perfusion chamber. Circulation of fluid inside each half chamber was induced immediately by bubbling the same gas mixture through at a rate of three to five bubbles/sec to maintain the solution at a constant pH of 7.65.

Samples ranged from 0.1 to 0.5 ml depending on the assay sensitivity of each drug. Samples were withdrawn from the receiving chamber (i.e., endothelial side) over a 4-hr period. An equal volume of solution was immediately added to the receiving solution to maintain a constant volume. The first sample was withdrawn within 2 min after starting the permeation and served as a control to detect leakage and rapid penetration. Subsequent samples were taken approximately every 40 min through the 4-hr period.

The sampling method for the corneal permeability experiments of levbunolol and nadolol varied from other drugs in that equal volumes of solutions (0.1 ml) were removed from both sides of cornea. In this way, equal volumes on both sides were maintained throughout the experiment.

After each permeability experiment, the cornea was trimmed of excess scleral tissue and conjunctiva, weighed, and dried in an oven overnight at 103°. After each cornea was dried, it was reweighed so that the hydration level of the wet cornea could be determined. The normal cornea has a hydration level of 76–80% (18). If manipulation of the cornea or if the drug itself led to damage of the epithelium and/or endothelium, then the hydration level would rise (83–92%) and the data were discarded.

Determination of Aqueous Diffusional Layer Resistance in the Perfusion Chamber—Fluid circulation in the chamber was provided

Table II—Experimental Conditions for the HPLC Assay of β-Blocking Agents

Drug	Column[a]	Wavelength, nm	Methanol, %[b]	Flow Rate, ml/min
Acebutolol	A	254	30, 38	2
Atenolol	A	254	20, 7.5	2
Bevantolol	B	254	28, 30	2
Bufuralol	B	254	28, 30	2
Levbunolol	B	254	28, 47	2
Metoprolol	A	280	35, 35	2
Nadolol	A	254	20, 31	2
Oxprenolol	B	280	22, 15	2.5
Penbutolol	B	254	25, 62.2	2
Propranolol	B	254	28, 23	2
Sotalol	A	254	42°, 30	2.5
Timolol	A	254	42°, 30	2.5

[a] (A) μ-Bondapak C18; (B) μ-Bondapak CN, Waters Associates, Milford, Mass. [b] The two numbers represent the percentage of methanol in the mobile phase for partitioning and corneal permeability determinations, respectively; the aqueous phase contained 1.5% acetic acid and was adjusted to pH 4 by sodium hydroxide. [c] The aqueous phase consisted of 58% 0.005 *M* heptanesulfonic acid and 1% acetic acid; the flow rate was 2.0 ml/min for these conditions.

[14] Morrison Rabbitry, West Branch, Iowa.

[15] Medical Research Instruments, University of Iowa, Iowa City, Iowa.

[16] Aldrich Chemical Co., Inc., Milwaukee, Wis.

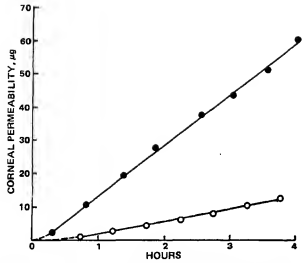


Figure 3—*Permeability rate of propranolol (●) and atenolol (○) across single excised rabbit corneas including linear regression lines; the initial concentrations for propranolol and atenolol were 85 and 2000 μg/ml, respectively.*

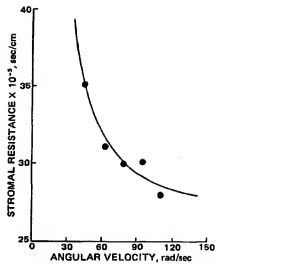


Figure 4—*Nonlinear regression results of the diffusional resistance of atenolol to stirring rate through excised rabbit stromal preparations. Each point is the average of 3–6 determinations; standard deviations were ≤10% of the mean.*

by maintaining a rate of three to five gas bubbles/sec. This maintains good mixing within each half chamber, as can be shown by adding a drop of colored solution into either half of the chamber and observing the homogeneous color that occurs in <1 min. However, to accurately determine the resistance of the cornea, it was necessary to detect and measure the magnitude of aqueous diffusional layer resistance, R_{aq}; consequently, the stirring was modified for these experiments.

The perfusion chamber was modified by installing two stirrers, one on each side of the cornea, with the center of each stirrer affixed 1 cm from the center of the corneal ring. Figure 2 depicts the modified perfusion chamber and rings used in mounting the cornea. In preliminary experiments it was observed that different rates of stirring induced varying degrees of swelling due to mechanical injury of the epithelium and endothelium.

An important purpose of the epithelium and endothelium is to control the thickness of the cornea by maintaining hydration levels at ~78%. By completely removing the epithelium and endothelium, the remaining stromal layer reached a constant and maximal thickness within the first 30 min of stirring such that subsequent changes in the stirring rate had no effect. The epithelium was removed by scraping with the blunt end of a scalpel blade. The endothelium was gently rubbed off with a cotton-tipped applicator (19). The removal of endothelium could be detected with the aid of a dissecting microscope. The epithelium was removed immediately following enucleation; the endothelium was removed just prior to mounting in the perfusion chamber. Atenolol (500 μg/ml) was chosen as the diffusing substance for these experiments. Since the aqueous diffusional barrier is independent of drug, these results were interpreted for the other β-blocking agents as well.

Once drug was placed adjacent to the cornea, the stirring speed was increased in steps every 30 min over a 4-hr period. The apparent permeability coefficients were determined for each 30-min increment. A sample was removed for atenolol analysis at the beginning and end of each 30-min period; both samples were used to calculate the permeability coefficient for each stirring speed. To minimize biological variability, each cornea was used to generate five or six permeability coefficients over a period of 4 hr.

Drug Assay—An HPLC method was used for analysis of each drug. The HPLC system included an injector[17], solvent delivery system, UV-absorption detector[17], column[18], and recorder[19]. The injector was equipped with different-sized loops, ranging from 50 μl to 200 ml, which enabled the injection of an accurate volume of sample solution. A solution of known concentration was used as an external standard. Each sample solution was divided so that two injections could be made and the results averaged.

[17] Model 7125 injector; Rheodyne, Cotati, CA 94928.
[18] M-6000 A solvent delivery system, Model 440 absorbance detector, μ-Bondapak C18, and μ-Bondapak CN Columns; Waters Associates, Milford, MA 01757.
[19] Model 5211-1; OmniScribe; Houston Instruments, Austin, Tex.

The mobile phases consisted of varying ratios of methanol and deaerated, deionized water containing 1.5% acetic acid, adjusted to pH 4 using sodium hydroxide. One exception was the mobile phase for sotalol and timolol, which utilized 0.005 *M* heptanesulfonic acid (to increase retention time) and 1% acetic acid. Table II lists the types of columns used, wavelengths at which UV measurements were made, methanol content of the mobile phase, and mobile phase flow rate. Methanol percentages in the mobile phases varied depending on whether partitioning or permeability experiments had been conducted. Drug solutions withdrawn from the endothelial side following corneal permeability contain polar extracts from the cornea which were eluted from the column within 1–3 min. In all experiments the retention times for the drugs were between 4 and 12 min. Linearity existed over the concentrations employed for each β-blocking agent (correlation coefficients >0.99).

Calculation of Permeability Coefficients—The apparent permeability coefficient (P_{app}, cm/sec) was determined by (19):

$$P_{app} = \frac{\Delta Q}{\Delta t(3600)AC_0} \qquad \text{(Eq. 3)}$$

where the term $\Delta Q/\Delta t$ is the permeability rate (*i.e.*, steady-state flux, μg/hr) of drug across each excised cornea, C_0 is the initial drug concentration (μg/ml), *A* is the corneal surface area (cm²), and 3600 is the conversion of hours to seconds. Corrections of C_0 were made to account for the sample volume removed over time and subsequently replaced with blank solution.

The corneal thickness increases with hydration, and the permeability coefficient is inversely proportional to barrier thickness. Therefore, it was important to determine corneal thicknesses. For a 2-kg rabbit, the corneal thickness (*h*) can be determined by:

$$h\ (\text{cm}) = \frac{0.42 + \text{H}}{100} \qquad \text{(Eq. 4)}$$

where H is the mg of water/mg of dry tissue (20). When stromal thicknesses were swollen due to epithelial and endothelial removal, the stroma resistances, $R_{str,swl}$, that were used to assess the aqueous diffusional barrier were corrected to the normal stromal thickness as existing in intact cornea (*i.e.*, $R_{str,int}$) by:

$$R_{str,int} = R_{str,swl}\left(\frac{h_{int}}{h_{swl}}\right) \qquad \text{(Eq. 5)}$$

where subscripts int and swl represent intact cornea and swollen stroma, respectively.

RESULTS AND DISCUSSION

The pK_a and partition coefficients of each β-blocking agent are listed in Table I. Although the aromatic substituents varied substantially for the series, these were too far removed from the amino group to exert much

of an effect on the pK_a values. Therefore, most pK_a values were within a narrow range (8.97–9.65). The pK_a of bevantolol was slightly lower (8.38) because of the electron-withdrawing effect of the ethoxybenzyl substituent. Sotalol has two pK_a values, 8.15 and 9.72, which are close to one another and compare reasonably well to the values of 8.30 and 9.80 published by Garrett and Schnelle (21) using the potentiometric titration method at 25°. The anilino group in sotalol acts as a weak acid as a result of the electron-withdrawing effect of the neighboring sulfonyl group. Ionization of the anilino group accounts for spectral shifts (21) and correlates with the pK_a of 8.15. The second pK_a, 9.72, was then assigned to the protonated amine group in the alkyl side chain of sotalol.

The distribution coefficients obtained from the extraction method using octanol and Sorenson's buffer varied over a fourfold log range. The range in partitioning behavior of the series is a consequence of the differences in aromatic substitution. The partitioning results permitted the compounds to be grouped as very lipophilic, lipophilic, or hydrophilic, classifications which were predictable from structural considerations. In addition to the amino group, the hydrophilic compounds also contained relatively polar substituents on the aromatic ring. Therefore, hydrogen bonding interactions with water are greater for atenolol and acebutolol, which contain amido groups, for nadolol, which contains a dihydroxy function and for sotalol, which contains a sulfonamido moiety. The very lipophilic compounds, on the other hand, contain hydrophobic substituents. The cyclopentyl group on the benzene ring gives penbutolol a high distribution and partition coefficient, whereas the furanyl group imparts lipophilicity to bufuralol. The ethoxybenzyl substituent in bevantolol not only lowers its pK_a, but also increases its lipophilicity. The high partition coefficient of propranolol is a result of the high lipophilic contribution of naphthalene. Based on the partitioning results of the very lipophilic and hydrophilic compounds, the remaining compounds (levbunolol, oxyprenolol, labetalol, and metoprolol) appear to contain substituents of an intermediate nature as far as polarity.

Corneal Permeability—The permeability coefficients of each β-blocking agent were obtained by linear regression of the steady-state flux. Figure 3 shows a plot of Q *versus* t for propranolol and atenolol across excised rabbit corneas. The data points closely fit the least-squares regression line once steady state has been reached. The lag time, defined by the linear intercept on the time axis, is related to the time required to reach steady-state permeation; more specifically, it is inversely related to the permeability coefficient. Consequently, the more rapidly penetrating compounds will have a shorter lag time and a greater steady-state flux. The slope of the straight line ($\Delta Q/\Delta t$), was substituted into Eq. 3 to obtain the apparent permeability coefficient. The apparent permeability coefficient also contains any aqueous diffusional layers that may exist on each side of the cornea.

Mathematical Model Relating Stirring Rate to Aqueous Diffusional Layer Resistance—The total diffusional resistance, R_{app}, through a multilayered barrier is represented by (22):

$$R_{app} = \frac{1}{P_{app}} = \sum_{i=1}^{n} R_i = \sum \frac{h_i}{D_i A(\mathrm{PC})_i} \qquad \text{(Eq. 6)}$$

where i represents each homogeneous barrier in series, n represents the total number of barriers, h represents barrier thickness, A represents surface area, D represents the diffusion coefficient, and PC represents the partition coefficient (22). With regard to significant diffusional layers, the rabbit cornea possesses two main tissue types: the lipophilic epithelium and endothelium, and the hydrophilic stroma. Assuming the existence of an aqueous diffusional barrier, the apparent resistance of the cornea can be considered as layers in series (23, 24) or:

$$R_{app} = \frac{1}{P_{app}} = R_{epi} + R_{str} + R_{endo} + R_{aq} \qquad \text{(Eq. 7)}$$

or

$$R_{app} = R_T + R_{aq} \qquad \text{(Eq. 8)}$$

where R_{aq} represents the sum of aqueous diffusional resistances on each side of the cornea and R_T represents the sum of the resistances of the corneal layers (epithelium, stroma, and endothelium).

According to the Nernst theory (25), there is a thin layer of static liquid of thickness h_{aq} immediately adjacent to a solid body. Outside of the static liquid layer is the well-stirred bulk solution. Experimental determinations have shown that the aqueous diffusional layer thickness, h_{aq}, can be expressed as:

$$h_{aq} = V^{-n} \qquad \text{(Eq. 9)}$$

where V is the velocity of the moving liquid. The exponent n depends on the experimental conditions ranging from $n = 0.33$ to ≥ 1. The thickness measurements representing the diffusional layer are apparent and not real. For example, experimental measurements have shown that the liquid retains its mobility down to a distance from the solid surface smaller than h_{aq}. Despite the fact that the Nernst theory may not exactly represent the diffusional behavior at the interface of liquid and solid, it can be used empirically to calculate R_{aq} (25).

In determining the resistance of the β-blocking agents across excised rabbit corneas within the stirred perfusion chamber, the following equation, which combines Eqs. 6–8, was considered:

$$R_{app} = R_{str} + \frac{h_{aq1}}{DA(\mathrm{PC})} + \frac{h_{aq2}}{DA(\mathrm{PC})} \qquad \text{(Eq. 10)}$$

where h_{aq1} and h_{aq2} are the aqueous diffusional layer thicknesses on each side of the mounted cornea at a given stirring rate, and R_{str} represents the membrane resistance for the stroma. R_{app} is measured experimentally at a specific stirring rate, *i.e.*, $1/P_{app}$.

By assigning $h_{aq} = h_{aq1} + h_{aq2}$ and substituting V^{-n} for h_{aq}, then Eq. 10 can be expressed as:

$$R_{app} = R_{str} + \frac{V^{-n}}{DA(\mathrm{PC})} \qquad \text{(Eq. 11)}$$

In the modified perfusion chamber, the stirrer is at the center of a circle 1 cm in diameter which contacts tangentially with the membrane and the perfusion chamber wall. Assuming that the liquid velocity tangential to the membrane, V, is proportional to the angular velocity of stirring, v, then Eq. 9 becomes:

$$R_{app} = R_{str} + \frac{(mv)^{-n}}{DA(\mathrm{PC})} \qquad \text{(Eq. 12)}$$

where m is a proportionality factor between the liquid velocity (cm/sec) and the angular velocity (rad/sec)[20]. To perform the nonlinear regression analysis the diffusion coefficient (D) was approximated by 1×10^{-5} cm^2/sec, an appropriate estimate for compounds of 200–300 molecular weight (22); PC was assigned a value of 1 for the aqueous system, and A was assigned a value 1.087 cm^2, which represented the surface area of the cornea used throughout the study. The remaining unknown parameter values (R_T, m, and n) were determined from the nonlinear regression analysis[21]. Figure 4 shows the results for atenolol permeation through excised stromal preparations with stirring rates varying from 425 to 1050 rpm. The computer-generated parameter values substituted into Eq. 10 become:

$$R_{app} = 26.8 \times 10^3 + 100{,}000\,(0.1006v)^{-1.67} \qquad \text{(Eq. 13)}$$

where 26.8×10^3 sec/cm represents the intrinsic stromal resistance, R_{str}. The apparent stromal resistance to atenolol permeation was 30.5×10^3 sec/cm. This latter value represents the experimental conditions for the perfusion chamber when stirred with the bubbling action of O_2–CO_2. The difference between the two resistances ($R_{app} - R_{str}$) is 3.7×10^3 sec/cm and represents the aqueous diffusional layer resistance, R_{aq}. This value was used in determining the intrinsic membrane resistances for the other β-blocking agents.

Permeability *versus* Partitioning Correlations—Figure 5 shows a plot of log P_T[22] against log PC; Table 111 contains the calculated parameter values. The data, although somewhat scattered, shows a plateau region for the very lipophilic compounds (propranolol, bufuralol, bevantolol, and penbutolol). The rate-determining factor responsible for the plateau region is not a result of the aqueous diffusion layer, since its contribution was subtracted from the experimentally determined permeability coefficients. The permeability rate is probably controlled by the hydrophilic stroma for these very lipophilic compounds. The relatively poor permeability shown for the hydrophilic compounds nadolol and sotalol possibly explains their poor potential for lowering intraocular pressure.

Multiple regression analyses[23] (26) were performed on the data to find the best set of parameters to describe the change in log P_T with a change in either log PC or log DC. Although the ranges in molecular weight and pK_a were relatively narrow for the β-blocking agents selected for study,

[20] rpm was converted to rad/sec by: rad/sec = rpm(2π)/60.

[21] Nonlinear regression was performed using the BMDP3R programs on an IBM370, at the University of Iowa Computer Center, University of Iowa, Iowa City, IA 52242.

[22] P_T represents the permeability coefficient across the intact excised rabbit cornea; $P_T = 1/R_T$.

[23] Multiple linear regression was performed using the BMDP1R, BMDP2R, and BMDP9R regression programs on an IBM370 computer.

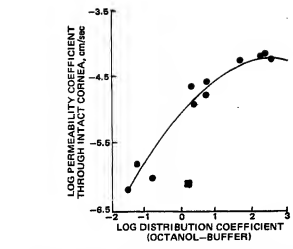


Figure 5—*Log–log plot of permeability coefficient (pH 7.65) and distribution coefficient (pH 7.65). The regression curve is represented by:* $\log P_T = 0.623 \log DC - 0.108 (\log DC)^2 - 5.0268$, *where* $r = 0.9756$ *and* $n = 11$; *acebutolol (■) is included in the figure, but not in the regression curve.*

a log MW term and a log DI[24] term were included in the analysis. Molecular weight (MW) is inversely related to diffusion and has been shown to improve correlations of this type (27, 28). Because of the plateau region (Fig. 5), a (log PC)^2 or (log DC)^2 term was also included. All possible subsets were analyzed, beginning with the intercept plus one parameter, then the intercept plus two parameters, *etc.*, up to the single set representing the intercept plus the maximum of four parameters. The correlation coefficient (r) and systematic deviation were used as the criteria to judge the best fit. The best fit for log P_T was represented as a function of all the parameters:

$$\log P_T = 1.01 \log PC - 0.115(\log PC)^2 - 5.64 \log MW - 10.4 \log DI + 7.27 \quad \text{(Eq. 14)}$$

$$r = 0.9272 \qquad p = 0.0041 \qquad n = 12$$

Both molecular weight and degree of ionization showed the expected inverse relationship to permeability. However, with either the molecular weight or degree of ionization term omitted from the regression analysis, the correlation coefficient was reduced only minimally to 0.9369 or 0.9678, respectively. With both molecular weight and degree of ionization removed, the correlation coefficient was 0.8560. With only the log PC term and the intercept, the regression analysis yielded a correlation coefficient of 0.8523. This latter linear regression line, however, shows systematic deviation at the plateau region and was not considered an acceptable fit to the data.

When DC was substituted for PC the multiple regression analyses produced an equally good fit:

$$\log P_T = 0.681 \log DC - 0.123(\log DC)^2 - 5.04 \log MW - 2.64 \log DI + 7.22 \quad \text{(Eq. 15)}$$

$$r = 0.9282 \qquad p = 0.0040 \qquad n = 12$$

When the degree of ionization was removed from consideration in Eq. 15, the correlation coefficient was reduced slightly to 0.9223. The lack of improvement from considering the degree of ionization is understandable, since the distribution and permeability coefficients represent the data at the same pH. By excluding the molecular weight term and (log DC)^2, the correlation coefficient was 0.8908 illustrating the small, but necessary, contribution of the squared term when systematic deviation is considered.

The hydrophilic acebutolol deviated the most from any regression line. By considering acebutolol as an outlier and excluding it from the regression analysis, the correlation coefficients increased. For example, the best regression lines yielded:

$$\log P_T = 0.972 \log PC - 0.112(\log PC)^2 - 2.71 \log MW - 9.26 \log DI + 0.219 \quad \text{(Eq. 16)}$$

[24] DI represents degree of ionization and was calculated from: $DI = 1/[1 + \text{antilog}(pH - pK_a)]$.

Table III—Permeability Coefficients and Physical Constants of β-Blocking Agents[a]

Drug	log P_T, cm/sec	log DC	log PC	log MW	log DI
Penbutolol	−4.22	2.53	4.15	2.46	−0.0106
Bufuralol	−4.14	2.31	3.65	2.44	−0.0200
Bevantolol	−4.17	2.19	3.00	2.50	−0.0740
Propranolol	−4.24	1.62	3.21	2.41	−0.0114
Levbunolol	−4.76	0.72	2.40	2.51	−0.0092
Oxprenolol	−4.56	0.69	2.37	2.42	−0.0092
Timolol	−4.91	0.34	1.91	2.49	−0.0119
Metoprolol	−4.62	0.28	1.88	2.50	−0.0110
Acebutolol	−6.07	0.20	1.77	2.52	−0.0119
Nadolol	−5.99	−0.82	0.93	2.49	−0.0079
Sotalol	−5.79	−1.25	−0.62	2.43	−0.0040
Atenolol	−6.17	−1.52	0.16	2.42	−0.0092

[a] P_T represents the permeability coefficient across the intact excised rabbit cornea; DC represents distribution coefficient; PC represents partition coefficient; MW represents molecular weight; DI represents degree of ionization.

$$r = 0.9696 \qquad p = 0.0008 \qquad n = 11$$

$$\log P_T = 0.623 \log DC - 0.108(\log DC)^2 - 5.0268 \quad \text{(Eq. 17)}$$

$$r = 0.9756 \qquad p < 0.00009 \qquad n = 11$$

Equation 17 predicts an optimum log DC value of 2.88, determined by setting $d \log P_T/d \log DC$ equal to zero and solving for log DC. However, there is no experimental evidence that a parabola would best describe the data. Compounds of greater lipophilicity than penbutolol could not be obtained to test this phenomenon.

Although correlations of this type are helpful in predicting useful molecular modifications, extrapolation to *in vivo* ophthalmic bioavailability must take into consideration solubility, the short residence time of instilled drops in the eye, and rapid metabolism or poor distribution to the target tissue. For example, a drug may have ideal partitioning behavior, but if it is not soluble, its concentration in tears will be too low to achieve an adequate penetration rate since the penetration rate is equal to the permeability coefficient multiplied by tear concentration. If a suspension is formulated because of the poor drug solubility, expulsion of the particles by the eye may take place before solubilization occurs, resulting in lower bioavailability.

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Corneal Penetration Behavior of β-Blocking Agents II: Assessment of Barrier Contributions

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Received July 26, 1982, from the *Pharmaceutics Division, College of Pharmacy, University of Iowa, Iowa City, IA 52242.* Accepted for publication September 16, 1982. *Present Address: National Defense Medical Center, P.O. Box 8244-14, Taipei, Taiwan 107, ROC.

Abstract □ Rabbit corneas were excised and mounted in a chamber to determine the permeability characteristics of a group of β-blocking agents. By measuring the permeability rate of each drug across intact cornea, stroma alone, epithelium–stroma, and stroma–endothelium, it was possible to determine the resistance to penetration for each corneal layer. The reciprocal of the sum of resistances for the epithelium, stroma, and endothelium equaled the experimentally determined permeability coefficient for the intact cornea (104 ± 6.0%). Thus, the penetration of β-blocking agents through the excised rabbit cornea could be treated as three barriers in series. For hydrophilic compounds, the epithelium was the rate-determining barrier. The endothelium offered less resistance, whereas the stroma offered only very minimal resistance. The lipophilic compounds penetrated the excised cornea more rapidly. However, the stroma became rate-determining for the most lipophilic compounds (penbutolol, bufuralol, bevantolol, and propranolol). Although the octanol–buffer (pH 7.55) distribution coefficient of these compounds varied over a fourfold logarithmic range, the permeability coefficient was considered nearly constant [3.4 × 10^{-5} (±0.34) cm/sec] for stroma. Also, the ratios of tortuosity to porosity for the stromal layer were 1.58 ± 0.15. These results suggest that drug diffuses through an aqueous media of gel-like mucopolysaccharide interspersed by a matrix of collagen fibrils. From further analyses intra- and intercellular pathways for epithelium and endothelium were added to the model resulting in a sigmoidal representation of permeability coefficient *versus* distribution coefficient. However, the intercellular (pore) pathway could not be adequately quantified because of the variation in the data for very hydrophilic compounds.

Keyphrases □ β-Blocking agents—permeability characteristics, excised rabbit corneas, barrier contributions □ Permeability—β-blocking agents, excised rabbit corneas, barrier contributions □ Ophthalmic drugs—β-blocking agents, corneal permeability, rabbits, barrier contributions

To optimize the penetration rate of drugs across biological membranes, quantitative multiple regression analyses are conducted to relate permeability to various physicochemical factors (1–3). These factors are often related through a sum of log terms, including partition coefficient, molecular weight, and degree of ionization. With the use of a digital computer and the appropriate algorithms, the regression analysis can be performed by a stepwise addition or deletion of each term or by comparing all possible subsets of the terms (4). In this way the significance of each term can be ascertained. Once all relevant physiochemical properties have been defined, an optimal chemical structure can be proposed. This semi-empirical approach, however, does not characterize the biological limitations imposed by the membrane, such as the significance of parallel aqueous pore pathways or limiting diffusional layers.

The permeability coefficients (P_T) of 12 β-blocking agents through excised rabbit corneas mounted in a perfusion chamber at pH 7.65 were determined in the previous paper (5). Through multiple regression analyses (excluding one outlier), log P_T could be related to partitioning factors by:

$$\log P_T = 0.6228 \log DC - 0.1081(\log DC)^2 - 5.03$$
$$r = 0.9756 \quad p < 0.00009 \quad n = 11 \qquad \text{(Eq. 1)}$$

where DC represents the octanol–buffer (pH 7.65) distribution coefficient. Neither a log molecular weight term nor a log degree of ionization term significantly improved the correlation. The parabolic equation represented in Eq. 1 predicted optimal penetrability at a log DC value of 2.88, the apex of the parabola. However, the experimental data (log P_T *versus* log DC) was curvilinear, leveling off to a plateau such that the asymptotic transport model of Ho *et al.* (6) could be applied. It is the purpose of this study to determine the limiting biological factors governing the steady-state flux of β-blocking agents across the multilayered excised rabbit cornea.

EXPERIMENTAL

Drugs—β-Blocking agents used in the experiments were acebutolol hydrochloride[1], atenolol[2], bevantolol hydrochloride[3], bufuralol hydrochloride[4], levbunolol hydrochloride[3], metoprolol tartrate[5], nadolol[6],

[1] May & Baker LTD Research Laboratories.
[2] Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, Del.
[3] Warner-Lambert Co., Pharmaceutical Research Division, Ann Arbor, Mich.
[4] Roche Products LTD, Research Department.
[5] CIBA Pharmaceutical Co., Division of CIBA-GEIGY Corp., Summit, N.J.
[6] E. R. Squibb & Sons, Inc., Princeton, N.J.

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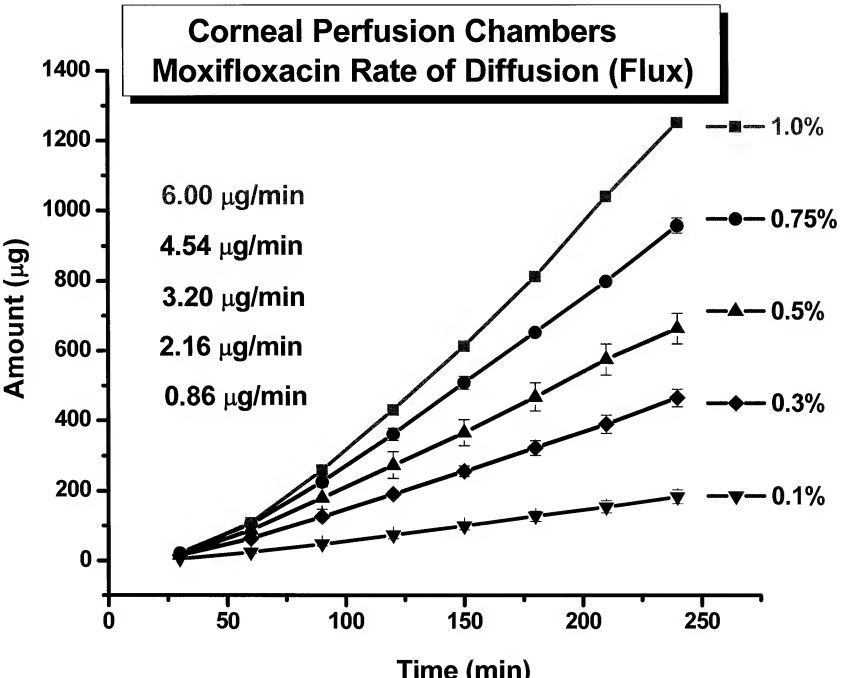
Corneal Perfusion Chambers
Moxifloxacin Rate of Diffusion (Flux)
Amount (µg)
Time (min)
1400
1200
1000
800
600
400
200
0
0
50
100
150
200
250
6.00 µg/min
4.54 µg/min
3.20 µg/min
2.16 µg/min
0.86 µg/min
1.0%
0.75%
0.5%
0.3%
0.1%

Corneal Perfusion Chambers
Rate of Diffusion (Flux) of Moxifloxacin

Concentration of Moxifloxacin	n	Slope (µg/min)	Total Accumulation after 240 min	Diffusion Coefficient Papp (10^{-7} cm/sec)	Lag Time (min)
0.1%	2	0.86	180	145	45
0.3%	2	2.16	455	121	29
0.5%	4	3.20	667	100	32
0.75%	2	4.54	930	102	35
1.0%	2	6.00	1197	101	41

CORNEAL PENETRATION AND CHANGES IN CORNEAL PERMEABILITY OF MOXIFLOXACIN VERSUS GATIFLOXACIN

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Abstract

Purpose: To compare the effects upon the cornea of moxifloxacin versus gatifloxacin by determining their corneal penetration and change in corneal epithelial permeability following exposure to fluoroquinolone solutions in ex vivo corneal perfusion models. Methods: The penetration studies were conducted with solutions of the fluoroquinolones (0.1%w/v), and the permeability studies were conducted with the commercial preparations: Vigamox™ (0.5% moxifloxacin, Alcon Laboratories, Inc.) which contains no BAC, and Zymar™ (0.3% gatifloxacin, Allergan, Inc.) which contains 0.003% BAC. In both sets of evaluations, corneas of NZW rabbits were excised and mounted in corneal perfusion chambers according to established methods. The penetration studies were conducted with the fluoroquinolone solutions applied to the epithelial side of the cornea. The rates of accumulation of the fluoroquinolones on the endothelial side of the chamber were determined using HPLC analysis of the perfusates over 5 hours. In the permeability studies, the commercial preparations were applied to the epithelial surface for 5 min. After rinsing, corneas were exposed to carboxyfluorescein (CF) for 5 min and the perfusate collected over 2 hrs. The level of CF in the perfusate was measured by spectrophotometry. Results: Moxifloxacin was found to have an apparent corneal permeability coefficient of 91 x 10^{-6} cm/sec, compared to 25 x 10^{-6} cm/sec for gatifloxacin. The lag time for the appearance of moxifloxacin on the endothelial side of the cornea was 49 min compared to 90 minutes for gatifloxacin. However, permeability of the cornea to CF was 2.1 pMol/min for Vigamox™ versus 3.4 pMol/min for Zymar™, with peaks of accumulation of 37 pMol/ml for Vigamox™ versus 60 pMol/ml for Zymar™. Conclusions: The apparent corneal penetration coefficient of moxifloxacin is 3.6 x greater than gatifloxacin and its appearance on the endothelial side is 2 x faster than gatifloxacin in the absence of any penetration enhancers such as BAC. Although the drug penetration is greater, the corneal permeability to CF is 1.8 x lower after exposure to Vigamox™ compared to Zymar™, illustrating that Vigamox™ maintains better corneal integrity. This demonstrates that the superior corneal penetration of moxifloxacin is due to the inherent characteristics of the moxifloxacin molecule, and is not due to changes in the corneal epithelial intercellular tight junctions.

Introduction

Moxifloxacin and gatifloxacin are both fourth generation fluoroquinolones that have recently been approved for the treatment of bacterial conjunctivitis. They have a broad spectrum of activity, good solubility, and are less likely to develop bacterial resistance because of their dual mode of action. The purpose of the present study is to determine and compare:

- The corneal penetration of these two fluoroquinolones using an *ex vivo* rabbit cornea under steady state conditions.
- The corneal permeation after a 5 min exposure to the commercial preparations: VIGAMOX™ (0.5% moxifloxacin, Alcon Laboratories, Inc.) and ZYMAR™ (0.3% gatifloxacin, Allergan, Inc.) ophthalmic solutions.
- Accompanying changes in corneal epithelial permeability.

Methods

Female New Zealand Albino rabbits weighing about 3 kg were handled and housed in accordance with USDA, AALAC and ARVO Regulations & Standards. After sacrifice, the intact eyes, along with the lids and conjunctival sacs were enucleated and immediately stored in fresh BSS PLUS® Irrigating Solution saturated with O_2/CO_2 (95:5).

- In the penetration experiments, within one hour, corneas were mounted in the modified perfusion chamber as described by Schoenwald (1). In the first series of experiments, 0.1 mMole solutions of the fluoroquinolones were prepared in the BSS solution and applied to the epithelial side of the cornea. Samples were withdrawn from the BSS containing endothelial side of the chamber over a period of five hours, and the concentration of fluoroquinolones was determined using HPLC analysis. In the second series of experiments, the commercial preparations VIGAMOX™ and ZYMAR™ were applied to the epithelial side of the cornea for 5 min followed by rinsing. Again, samples were withdrawn from the endothelial side and analyzed over a period of five hours.
- In the permeability experiments, the corneas were mounted in perfusion cells as described by Weisman (2). After a one-hour perfusion with BSS solution, the commercial preparations VIGAMOX™ and ZYMAR™ were applied to the epithelial side of the cornea for 5 min followed by rinsing, and then 6 5 min exposure to carboxyfluorescein (CF). The perfusate was collected from the endothelial side of the cornea over a two-hour period, and the level of CF was determined using luminescence spectrophotometry.

Results

Under steady state conditions in BSS (Figure 1 and Table 1, n = 3), moxifloxacin was found to have an apparent corneal permeability coefficient (1) of 91 x 10^{-6} cm/sec, compared to 25 x 10^{-6} cm/sec for gatifloxacin. The lag time for the appearance of moxifloxacin on the endothelial side of the cornea was 49 min compared to 90 min for gatifloxacin. Thus, moxifloxacin permeates the cornea at 3.6 times the rate of gatifloxacin, and appears on the endothelial side in half the time.

Figure 1. Corneal Penetration of Moxifloxacin and Gatifloxacin Under Steady State Conditions

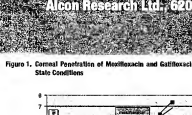

Figure 2. Corneal Penetration of Moxifloxacin and Gatifloxacin From Their Ophthalmic Solutions: VIGAMOX™ and ZYMAR™

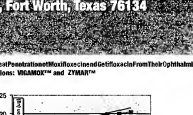

Figure 3. Corneal Permeability to Carboxyfluorescein (CF) After Exposure to: VIGAMOX™ and ZYMAR™

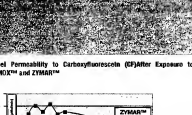

Table 1. Permeability Coefficients and Lag Times

Fluoroquinolone	Apparent Corneal Permeability Coefficient (x 10^{-6} cm/sec)	Lag Time (min)
Moxifloxacin	91 ± 9	49 ± 1
Gatifloxacin	25 ± 2	99 ± 12

Table 2. Rates of Accumulation and Lag Times

Fluoroquinolone Ophthalmic Solution	Rate of Accumulation µg/Lx(ml/m)	Lag Time (min)
VIGAMOX™	6.5 ± 1.6	11 ± 4
ZYMAR™	2.8 ± 0.3	18 ± 5

After a 5 min dosing regimen of their ophthalmic solutions, VIGAMOX™ and ZYMAR™ (Figure 2 and Table 2, n = 4), moxifloxacin was found to have a rate of accumulation on the endothelial side of the cornea of 6.5 µg/Lx(ml/m) compared to 2.8 µg/Lx(ml/m) for gatifloxacin. The lag times were 11 min for moxifloxacin and 18 min for gatifloxacin. So, from their ophthalmic formulations, moxifloxacin penetrates the cornea at 2.3 times the rate of gatifloxacin.

In the permeability experiments (Figure 3 and Table 3, n = 4), after a 5 min exposure regimen, the initial rate of permeability of the cornea to CF was 2.1 pMol/ml/min for VIGAMOX™ compared to 3.4 pMol/ml/min for ZYMAR™ with peaks of accumulation of 37 pMol/ml for VIGAMOX™ versus 60 pMol/ml for ZYMAR™. Thus VIGAMOX™ maintains superior corneal integrity by a factor of 1.6 compared to ZYMAR™.

Table 3. Rates and Peaks of Accumulation

Fluoroquinolone Ophthalmic Solution	Initial Rate of Accumulation (pMol/ml/min)	Peak of Accumulation (pMol/ml)
Control	0.8 ± 0.4	18 ± 8
VIGAMOX™	2.1 ± 0.6	37 ± 9
ZYMAR™	3.4 ± 0.6	60 ± 5

Discussion

The concentration of moxifloxacin in VIGAMOX™ is 0.5%, and the concentration of gatifloxacin in ZYMAR™ is 0.3%. Based upon the corneal penetration results under steady state conditions, the expected advantage of moxifloxacin over gatifloxacin is 6 times (3.6 x 0.5/0.3). However, with the ophthalmic formulations, the observed advantage for moxifloxacin is 2.3 times. This suggests that the vehicle formulation of ZYMAR™ may be compromising the corneal integrity. The permeability results with CF, confirm that VIGAMOX™ maintains superior corneal integrity compared to ZYMAR™.

Conclusions

- The *ex vivo* corneal penetration of moxifloxacin in BSS solution is 3.6 times greater than gatifloxacin. In addition, moxifloxacin appears on the endothelial side of the cornea 2 times faster than gatifloxacin.
- The penetration of moxifloxacin is 2.3 times greater than gatifloxacin when using a 5 min dosing regimen of the fluoroquinolones from their marketed formulations: VIGAMOX™ and ZYMAR™.
- However, the corneal permeability to carboxyfluorescein is 1.6 times lower for VIGAMOX™ compared to ZYMAR™. This demonstrates that VIGAMOX™ maintains superior corneal integrity compared to ZYMAR™. Thus, the superior corneal penetration of moxifloxacin is due to the inherent characteristics of the moxifloxacin molecule, and is not due to changes in the corneal epithelial intercellular tight junctions.

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APPENDIX H

Aqueous Penetration and Biological Activity of Moxifloxacin 0.5% Ophthalmic Solution and Gatifloxacin 0.3% Solution in Cataract Surgery Patients

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Purpose: To measure the achievable perioperative aqueous concentration of the commercially available topically administered fourth generation fluoroquinolones, moxifloxacin 0.5% ophthalmic solution, and gatifloxacin 0.3% ophthalmic solution, and to correlate this concentration with the agents' biological efficacy in the aqueous humor of patients undergoing routine cataract surgery.

Design: Prospective, randomized, parallel, double-masked, clinical trial.

Participants: Fifty patients undergoing cataract extraction.

Methods: Patients (n = 25) were given perioperative topical moxifloxacin 0.5% or topical gatifloxacin 0.3% (n = 25). One drop of antibiotic was administered every 10 minutes for 4 doses beginning 1 hour prior to surgery. Aqueous humor was sampled via paracentesis and antibiotic concentrations were determined using validated high performance liquid chromatography (HPLC) procedures. Dilution analyses were performed to determine the biological efficacy of the agents in the aqueous against *Staphylococcus epidermidis*, the most common cause of postcataract endophthalmitis.

Main Outcome Measures: Aqueous humor antibiotic concentrations were measured using HPLC and microdilution bioassay techniques. Biological activity was measured as minimal inhibitory dilution and minimal bactericidal dilution.

Results: Aqueous humor concentrations for moxifloxacin via HPLC analysis were 1.80 (±1.21) μg/ml, whereas those for gatifloxacin were 0.48 (±0.34) μg/ml. This 3.8-fold difference in aqueous humor antibiotic concentrations was statistically significant ($P = 0.00003$). Similarly, the biological dilution analysis of the aqueous humor samples showed that moxifloxacin attained an estimated activity of 2.1 μg/ml, whereas the gatifloxacin activity was approximately 0.4 μg/ml, which represented a 4.9-fold difference.

Conclusions: This study demonstrated that after topically administered perioperative antibiotics with cataract surgery, moxifloxacin 0.5% ophthalmic solution achieved a statistically significantly higher concentration in aqueous humor compared with gatifloxacin ($P = 0.00003$). Results from the broth dilution analysis showed that moxifloxacin 0.5% was biologically more active against *S. epidermidis* than gatifloxacin 0.3% in aqueous humor after topical application. There were no adverse events reported, and incision wounds healed quickly and as expected. *Ophthalmology 2005;112:1992–1996 © 2005 by the American Academy of Ophthalmology.*

Recent reports indicate that endophthalmitis rates after cataract surgery are on the rise (McDonnell PJ. Endophthalmitis risk factors: clear corneal incision? Presented at: American Academy of Ophthalmology meeting, October 25, 2004; New Orleans, Louisiana). The outcome of this intraocular infection can be devastating to the patient and result in significant loss of vision and even loss of the eye. Goals of perioperative administration of antibiotics are to prevent infection by decreasing colonization of the ocular surface normal flora and pathogens as well as to achieve a therapeutic intraocular antibiotic concentration.[1–3] Gram-positive pathogens are the most common organisms implicated in endophthalmitis cases.[4] Recently, there has been an emergence of resistant gram-positive organisms recovered from cases of endophthalmitis.[5–12]

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Portions of this work were presented at: Association for Research in Vision and Ophthalmology meeting, May 5, 2005; Fort Lauderdale, Florida.

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APPENDIX I

The fourth generation fluoroquinolone eyedrops have been developed to broaden the spectrum of antibiotic coverage, including resistant strains. These molecules have a lower propensity to promote the development of resistance, because they require 2 mutations to establish resistance, one in the topoisomerase IV gene and a second one in the DNA gyrase (topoisomerase II) gene.[6,7] In addition, the bulky C-7 substituent of the moxifloxacin molecule renders it a poor substrate for bacterial efflux pumps, which effectively prevents it from being removed from the bacterial cell.[13] Therefore, more of the antibiotic accumulates within the bacterial cell resulting in rapid bacterial cell death. Gatifloxacin contains a methoxy substituent at position 8 of the quinolone ring that has been associated in some bacteria with increased bacteriostatic and bactericidal activity, as well as decreased selection of resistant mutants.[14]

In vivo penetration studies have been conducted with all of the ophthalmic fluoroquinolones: both second generation fluoroquinolones, ciprofloxacin and ofloxacin, the third generation fluoroquinolone, levofloxacin, as well as with the fourth generation fluoroquinolones, moxifloxacin and gatifloxacin (Invest Ophthalmol Vis Sci 44[suppl]:1454, 2003; Invest Ophthalmol Vis Sci 44[suppl]:2117, 2003; Invest Ophthalmol Vis Sci 45[suppl]:4906, 2004).[15,16] In studies with human patients, it has been demonstrated that moxifloxacin can safely penetrate the cornea and achieve a higher concentration in the aqueous at least 2 to 3 times that of other fluoroquinolones (Invest Ophthalmol Vis Sci 46[suppl]:5051, 2005).[17–19]

The purpose of this study was to measure the perioperative aqueous concentration of the fourth generation fluoroquinolones, moxifloxacin, and gatifloxacin and to correlate this concentration with the biological activity of the agent within the aqueous specimen against the most common endophthalmitis-causing organism, *S. epidermidis*. This biological activity took into account the protein binding and other host factors that could affect the in vivo activity of the fluoroquinolone. To our knowledge, this is the first in vivo study to look at both the achievable concentration and the relative biological activity of these 2 newer generation 8-methoxy fluoroquinolone topical ocular antibiotics. Therefore, we present 2 different assessments of penetration and biological efficacy from aqueous humor samples in patients undergoing cataract surgery, both corroborating the same surgical outcome of higher potency and penetration with moxifloxacin 0.5% ophthalmic solution.

Patients and Methods

This was a prospective, randomized, parallel, double-masked, clinical trial involving 50 patients undergoing cataract extraction who were given perioperative topical moxifloxacin 0.5% (Vigamox, Alcon Laboratories, Inc., Fort Worth, TX; n = 25) or gatifloxacin 0.3% (Zymar, Allergan Inc., Irvine, CA; n = 25) commercially-available ophthalmic solutions. Institutional Review Board/Ethics Committee approval was obtained. Surgical methods were previously described in a preliminary report.[20] On the day of surgery, patients were randomly assigned to receive drops 10 minutes apart for a total of 4 doses with the last dose given (±2) minutes prior to the time of initiating the cataract incision. A 15-degree superblade was used to make a paracentesis, and a 30-gauge cannula on a tuberculin syringe then was used to acquire the aqueous specimen immediately through the paracentesis site. Once the specimen was acquired, it was transferred immediately to an Eppendorf tube using sterile gloves and stored in –70° C.

Moxifloxacin and gatifloxacin concentrations in human aqueous humor were determined by an independent laboratory using a validated high performance liquid chromatography (HPLC)-tandem mass spectrometry method as previously described.[18] Briefly, 50 μl of human aqueous humor was spiked with a tetra-deuterated moxifloxacin internal standard. The samples were prepared using reversed-phase, solid-phase extraction cartridges. The HPLC was performed on a reversed-phase C8 column.

Broth Dilution Assay

The bactericidal activity of the aqueous humor was determined according to the National Committee for Clinical Laboratory Standards guideline for performance of the serum bactericidal test.[21] This methodology is useful in assessing the inhibitory and lethal activity of antibiotics in vivo, because it takes into consideration host factors such as penetration and protein binding as well as organism–antibiotic interaction. For analysis, 0.1 ml of each aqueous humor sample was serially diluted 2-fold in 0.1 ml of cat ion adjusted Mueller–Hinton broth in sterile microtiter plates over a dilution range of 1:2 through 1:128. The reference organism used to determine the bactericidal activity of the aqueous samples was a clinical isolate of *S. epidermidis*, the most common causative organism for postcataract endophthalmitis. The reference *S. epidermidis* utilized in these experiments exhibited a minimal inhibitory concentration (MIC) of 0.1 μg/ml to gatifloxacin and an MIC of 0.05μg/ml to moxifloxacin. These MIC values are consistent with those found in the published literature.[6] For analysis, the reference strain was grown overnight on trypticase soy agar with 5% sheep blood, 3 to 5 colonies were inoculated into CAMHB, and incubated for 6 hours at 35° C, and then the inoculum broth culture was adjusted to 0.5 MacFarland standard (~$1–5\times10^8$ CFU/ml) diluted 1:10 in CAMHB and 0.01 ml and was added to each microtiter well to yield a final concentration of $1–5\times10^4$) CFU/microtiter well. Trays were incubated in ambient air at 35° C for 24 hours. Each specimen was read for turbidity and the highest dilution demonstrating no growth was determined as the minimal inhibitory dilution. Wells showing no turbidity were quantitatively (0.1 ml) subcultured to trypticase soy agar with 5% sheep blood and incubated for 24 hours at 35° C. After incubation, the colonies were counted, and the minimal bactericidal dilution was determined as the highest dilution yielding ≤ 10 colonies (>99.9% Killing). For purposes of estimating the antibiotic concentration in each sample, the minimal inhibitory dilution was multiplied by the MIC of 0.05 μg/ml for moxifloxacin and 0.1 μg/ml for gatifloxacin of the reference *S. epidermidis*.

Statistical analyses were performed using a Student's *t* test to detect differences between the antibiotic treatment groups.

Results

Mean aqueous humor measured concentrations obtained via HPLC analysis for moxifloxacin were 1.80 (±1.21) μg/ml compared with for 0.48 (±0.34) μg/ml gatifloxacin (Fig 1). This represented a 3.8-fold difference in measured aqueous humor antibiotic concentrations, which was statistically significant ($P = 0.00003$).

Microbiological dilution bioassay of the aqueous humor samples showed that moxifloxacin attained an estimated antibiotic concentration based on inhibitory activity of 2.1 μg/ml (±1.7 μg/ml), whereas the gatifloxacin concentration was 0.4 μg/ml (±0.4

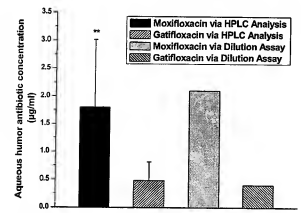

Figure 1. Aqueous humor antibiotic concentrations and dilution bioassay results from patients undergoing cataract surgery. One drop of antibiotic was administered every 10 minutes for 4 doses beginning 1 hour prior to surgery. The aqueous humor sample was collected at the time of incision within a 30 (±2) minute window from the time of the last antibiotic drop. Moxifloxacin achieved an aqueous humor concentration of 1.80 μg/ml, whereas gatifloxacin was 0.48 μg/ml. This difference was significantly different (**$P = 0.00003$). The aqueous humor concentrations were estimated as 2.1 μg/ml for moxifloxacin and 0.4 μg/ml for gatifloxacin by multiplying the minimal inhibitory dilution by the minimal inhibitory concentration of the reference *Staphylococcus epidermidis*. HPLC = high performance liquid chromatography.

μg/ml, Fig 1). Dilutional analysis resulted in an average minimal inhibitory dilution of 1:42 for moxifloxacin 0.5% and 1:4 for gatifloxacin 0.3% against *S. epidermidis*. The average minimal bactericidal dilution was 1:40 for moxifloxacin 0.5% ophthalmic solution and 1:3 for gatifloxacin 0.3% ophthalmic solution. Thus, moxifloxacin aqueous samples had to be diluted to a higher extent than gatifloxacin aqueous samples before bacterial growth was observed. These results showed that moxifloxacin 0.5% was biologically more active against *S. epidermidis* than gatifloxacin 0.3% in aqueous humor after topical application. There were no adverse events reported, and incision wounds healed quickly and as expected.

Discussion

Recent reports indicate that resistance to earlier generation ocular antibiotics among clinical bacterial isolates is becoming more prevalent.[2,7–12,22,23] The increasing number of ocular surgical procedures poses a greater risk for perioperative infection. Recent reports indicate an upward trend in the incidence of bacterial infections after cataract and refractive surgery.[24–26] This increased risk of surgical complications, such as postoperative endophthalmitis and keratitis, in part prompted the advance of the fourth-generation antibiotics moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% ophthalmic solution for the prevention and treatment of these potentially serious ocular infections. Moxifloxacin and gatifloxacin are 8-methoxy-substituted fluoroquinolone agents demonstrating greater potency against gram-positive organisms, and including for the first time, certain species of atypical mycobacteria compared with earlier generation fluoroquinolones while retaining excellent coverage against gram-negative bacteria.[6,7] Specific to endophthalmitis, Kowalski et al[27] demonstrated a first proof-of-principle for prophylactic topical fluoroquinolone antibiotic use in the prevention of endophthalmitis in an animal model demonstrating that pretreatment with moxifloxacin prevented development of endophthalmitis after a large intravitreal inoculum of bacteria administered to rabbits.

An important factor that contributes to the success of antibiotic therapy is the ability of the molecule to penetrate the target ocular tissues at concentrations greater than the MIC. The mutant prevention concentration is typically 8- to 10-fold higher than the MIC for a given organism.[28,29] Recent studies indicate the likelihood of selection for resistant microorganisms can be reduced by maintaining concentrations at or greater than the mutant prevention concentration.[30] Therefore, maintaining the highest possible ratio between aqueous humor fluoroquinolone concentrations and MIC, preferably at or greater than the mutant prevention concentration decreases the probability of selecting for single-step mutants.[7,31] Moxifloxacin 0.5% ophthalmic solution provided drug penetration at concentrations greater than the MICs for *S. epidermidis*, *Streptococcus pneumoniae*, *viridans streptococci*, *enterococci*, and *Bacillus* species, as well as fluoroquinolone-susceptible and resistant *Staphylococcus aureus* (Fig 2).[6] Gatifloxacin achieved MICs for all of these organisms, except for fluoroquinolone-resistant *S. aureus* (Fig 3).

The current penetration study corroborates reports from other investigators with animal models and human patients. Moxifloxacin (distribution coefficient at pH 7.4 = 0.61) is more lipophilic than gatifloxacin (distribution coefficient at pH 7.4 = 0.11) (Invest Ophthalmol Vis Sci 45[suppl]:4907, 2004). This facilitates the ability of moxifloxacin to traverse both the epithelial and endothelial corneal membrane layers. Tissue penetration studies with excised rabbit corneas demonstrated that moxifloxacin 0.5% safely produced a 3.6-fold

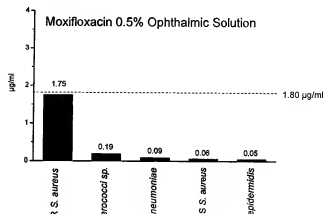

Figure 2. Moxifloxacin gram-positive minimal inhibitory concentrations in relation to aqueous humor concentrations from the current study. Minimal inhibitory concentration values are from Mather et al.[6] FQR = fluoroquinolone resistant; FQS = fluoroquinolone sensitive.

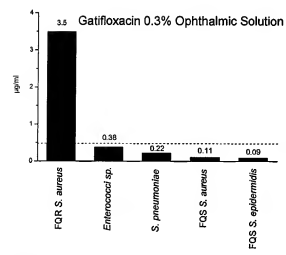

Figure 3. Gatifloxacin gram-positive minimal inhibitory concentrations in relation to aqueous humor concentrations from the current study. Minimal inhibitory concentration values are from Mather et al.[6] FQR = fluoroquinolone resistant; FQS = fluoroquinolone sensitive.

higher corneal penetration coefficient than gatifloxacin 0.3% (Invest Ophthalmol Vis Sci 45[suppl]:4910, 2004). The appearance of moxifloxacin on the endothelial side was also approximately 2-fold faster than gatifloxacin. Another rabbit study demonstrated that moxifloxacin was readily absorbed into the aqueous humor and anterior ocular tissues (Invest Ophthalmol Vis Sci 44[suppl]:1454, 2003). Solomon et al[17] conducted a prospective study in cataract patients in which the antibiotic dosing frequency was 4 times daily for 3 days prior to surgery. On the day of surgery, patients received the antibiotics every 15 minutes for 3 doses 1 hour prior to their procedure. Aqueous humor concentrations at the time of surgery were significantly higher for moxifloxacin ($P<0.05$) than for gatifloxacin. Katz et al[18] measured moxifloxacin aqueous humor concentrations in cataract patients who received 1 drop every 15 minutes for 4 doses before surgery (group 1), or 1 drop 4 times a day the day before surgery plus the same preoperative regimen as group 1 (group 2). The maximal concentration achieved with these 2 regimens was not significantly different. The area under the aqueous concentration-time curve ($AUC_{0-3\ hr}$) did show a difference in favor of group 2 ($P = 0.04$). This study also corroborates that topical moxifloxacin was well absorbed into the aqueous humor at concentrations much greater than the median MICs for common pathogens involved in endophthalmitis. In a prospective study of cataract patients dosed 4 times a day the day before surgery with 1 additional drop an hour before surgery, McCulley's group reported aqueous humor concentrations that were significantly higher for moxifloxacin than for gatifloxacin (Invest Ophthalmol Vis Sci 46[suppl]: 5051, 2005). The antibiotic concentrations attainable with topical dosing reported from these published studies are consistent with those from the current study that were measured via HPLC as well as through a microdilution bioassay.

To the best of our knowledge, there are no published studies that have compared the efficacy of achievable concentrations of moxifloxacin 0.5% ophthalmic solution with gatifloxacin 0.3% ophthalmic solution in aqueous humor via broth dilution.

Clinical studies with human patients confirm the preclinical studies with moxifloxacin and gatifloxacin that demonstrate that effective corneal penetration does not compromise the safety of these antibiotics (Invest Ophthalmol Vis Sci 46[suppl]:4903, 2005).[32–34] Yee et al's[35] group recently reported that there were no significant differences in human corneal wound healing, haze, or visual acuity between moxifloxacin 0.5% and gatifloxacin 0.3% (dosed every 6 hours until complete wound healing had occurred) for bilateral photorefractive keratectomy patients. Another study from the same laboratory showed equivalence between these 2 fluoroquinolone antibiotics (dosed 4 times a day for 3 days prior to surgery and 7 days postoperatively) in terms of flap clarity, stromal edema, flap edema, epithelial defect, and visual acuity for Epi-LASIK patients (Invest Ophthalmol Vis Sci 46[suppl]:4877, 2005). Durrie and Trattler[36] also reported that moxifloxacin 0.5% and gatifloxacin 0.3% ophthalmic solutions were equivalent in terms of corneal healing after LASIK and laser epithelial keratomileusis surgery. Thus, both products are believed to be biocompatible when administered in doses recommended by prevailing standards-of-care in a variety of ophthalmic surgical procedures.

The current study presents 2 assessments of fluoroquinolone penetration and biological efficacy from aqueous humor samples in patients undergoing cataract surgery. These results corroborate the same statistical and clinical outcomes of higher potency and therapeutic penetration for moxifloxacin 0.5% ophthalmic solution compared with gatifloxacin 0.3% ophthalmic solution. In our study, both moxifloxacin 0.5% and gatifloxacin 0.3% exceeded the known MIC values for most pathogens that cause endophthalmitis. The higher aqueous levels of moxifloxacin 0.5% may provide greater efficacy especially against fluoroquinolone resistant *S. aureus*.

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